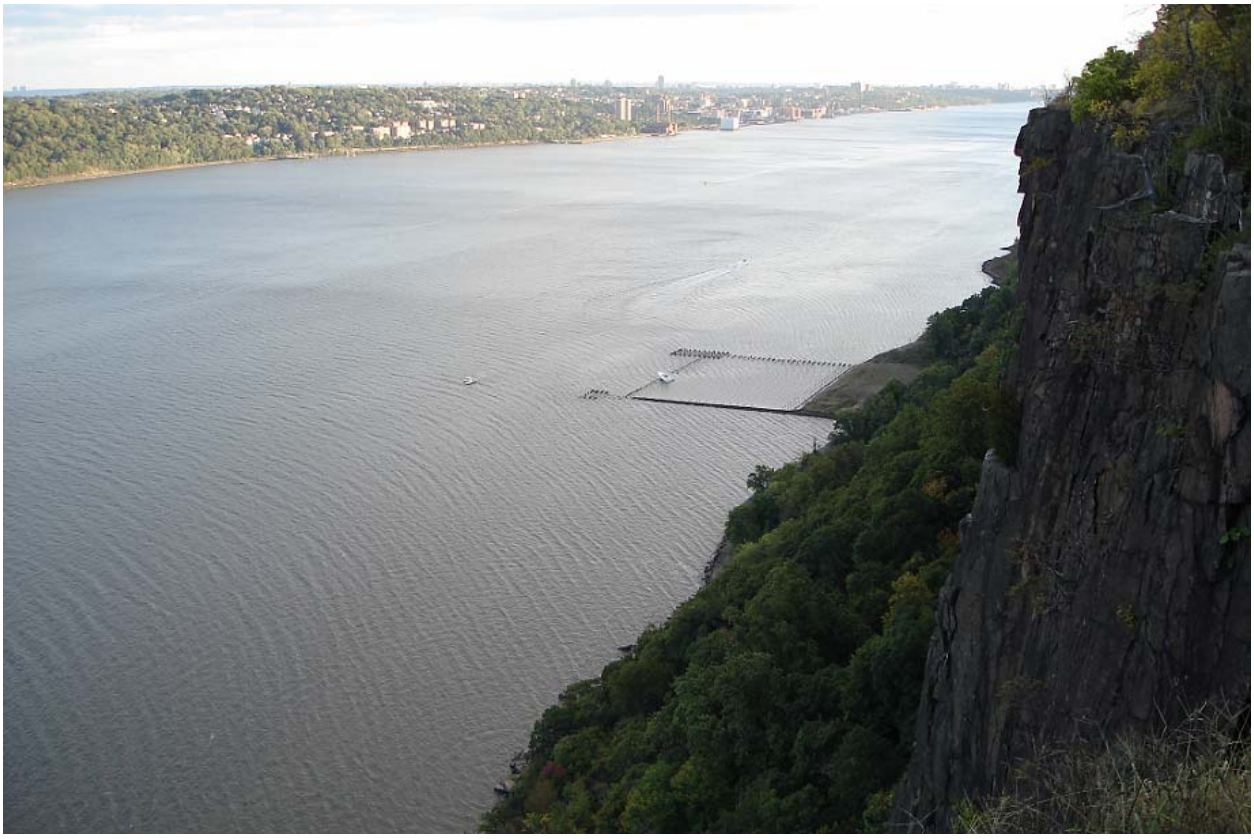
An International Journal

Nature and Science

ISSN 1545-0740

Volume 5 - Number 3 (Cumulated No. 16), September 10, 2007



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Lansing, Michigan 48909
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E-mail: editor@sciencepub.net,;
naturesciencej@gmail.com
Websites: http://www.sciencepub.org

Nature and Science

ISSN: 1545-0740

Volume 5 - Number 3 (Cumulated No. 16), September 10, 2007

Cover Page, Introduction, Contents, Call for Papers, All in one file

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Marsland Press, P.O. Box 21126, Lansing, Michigan 48909, The United States, (517) 349-2362

http://www.sciencepub.org editor@sciencepub.net

Comparative Leaf Epidermal Studies On *Solanum Macrocarpon And Solanum Nigrum*

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ABSTRACT: The comparative leaf epidermal studies on *Solanum macrocarpon* and *Solanum nigrum* showed that the upper and the lower epidermal cell wall of *S. nigrum* varied in shape from pentagonal, rectangular to hexagonal while the upper and the lower epidermal cell wall of *S. macrocarpon* are irregular in shape. Although both taxa are amphistomatic but stomata are more abundant at the lower leaf surfaces than the upper leaf surfaces. Both stomata are anomocytic with stomatal index ranging from 5.70%-33.02% in *S. nigrum* and 10.14%-32.35% in *S. macrocarpon*. These observations are important especially as they help to establish interspecie relationships among the two investigated taxa and hence suggest reasons for the two taxa to be in the same genus. [Nature and Science. 2007;5(3):1-4].

Keywords: Comparative, Leaf, epidermal studies, *Solanum,* species, Solanaceae

INTORDUCTION:

The genus *Solanum* belongs to the family Solanaceae. Members of this family are mostly herbs and twinners with about 70 genera and 2,000 specis (Willis, 1985). Some workers recorded about 85 genera and 2,200 species (Ahmed, 1964, Patel 1969). Solanaceae is represented in West Africa by 53 species contained in 8 genera (Hutchinson and Dalziel 1963*). S. macrocarpon* and *S. nigrum* are edible. They serve as foliage for feeding livestock but excess intake of *Solanum* plants especially those with bitter taste may lead to fruit toxicity and spinal bifida i.e. non joining of spinal bones due to ingestion of two much solanine. (Schippers, 2001).

From available literatures, the use of leaf epidermal features in systematic botany is now popular just like the use of other makers like DNA sequence and chemical compositions (Edeoga and Ikem, 2001; Mbagwu and Edeoga, 2006). Epidermal structures and stomatal ontogeny of some Nigerian ferns have been found relevant in their recognition (Gill and Karatela, 1985). Olowokwudejo (1990) compared the morphology of the leaf epidermis in *Annona* and suggested the utilization of this character in the identification of the species. Edeoga, 1991; Edeoga and Osawe, 1996; Mbagwu and Edeoga, 2006 constantly reaffirmed the point that epidermal and cuticular traits of plants could serve as vital tools exploitable in the systematics of the present day angiosperms. Also, different shapes of epidermal cells, type and arrangement of stomata, size and shape of trichomes and number of vascular bundles are all vital in systematic botany (Nwachukwu and Mbagwu, 2006). Perhaps the most extensive investigated family where anatomical features provided very useful taxonomic characters was the Gramineae and several authors have constructed keys for the identification of some taxa within the family based only on leaf epidermal characters (Davis, 1959). Edeoga and Osawe (1996) used the leaf epidermal morphology of some members of *Costus*, *Senna* and *Boerhavia* species to establish possible relationships among the different species they investigated.

Although the biological significance and implications of leaf anatomical characters have been highlighted in different plant families, there is no specific leaf anatomical documentation on *S. macrocarpum* and *S . nigrum* hence the need for this research investigation.

This paper therefore described the leaf epidermal characters of the two *Solanum* species. It also assesses the relevance of and discusses the extent to which leaf epidermal features might be utilized in the systematic consideration of the two species in view of their perceived similarities in structural and reproductive biology.

MATERIALS AND METHODS

Fresh leaves from the two *Solanum* species were collected. This work was done at the Crop Science Laboratory at University of Nigeria Nsukka in November, 2006. Epidermal peels were obtained directly from the fresh leaves without any chemical treatment. This was done by free hand peeling with razor blade. The epidermal peels obtained were stained with ethanol safranin for one minute. Excess safranin stain were

washed off and temporarily mounted in aqueous glycerol solution (Cutler, 1978). Photomicrographs of the epidermal features were taken from the slides using Letz Wetzler Ortholux microscope fitted with vivitar-v-335 camera. (Figure 1 a & b).

RESULTS AND DISCUSSION

The leaf epidermal features of the two *Solanum* species investigated were summarized in tables 1 and 2.The walls of the epidermal cells of the two species showed that the upper and the lower epidermal cell wall of *S. nigrum* varied in shape from pentagonal, rectangular to hexagonal while the upper and lower epidermal cell walls of *S. macrocarpum* are irregular in shape (Tables 1 and 2). The distribution of stomata in both the upper and lower epidermis also varied. This was apparent in the variation of the stomatal index ranging from 5.70% - 33.02% in *S. nigrum* and 10.14% - 32.35% in *S. macrocarpum*. Moreso the stomatal frequency varied in the lower epidermis of both taxa indicating 136.50% in *S. nigrum* and 137.40% in *S. macrocarpum*. Anomocytic stomata characterized the two species and both species are amphistomatic i.e. stomata are present in both the upper and lower epidermis (Tables 1 and 2).

Although stomata appeared on both the upper and lower leaf surfaces but they are more on the lower leaf epidermis. This is probably an adaptation to water loss. This is in agreement with Metacalf and Chalk (1950), Mbagwu and Edeoga (2006) who observed that stomata are present on both surfaces of leaf but are usually more on the lower epidermis in species of *Amaranthus* and *Vigna* respectively.The anomocytic type of stomata that characterized the two taxa is not strange since Edeoga and Ikem (2001) observed the same in *Boeheavia* species, Metcalfe and Chalk, (1960) observed the same in some dicotyledonous plants and Mbagwu and Edeoga (2006) also noticed the same in *Vigna* species. In each of this study, the authors emphasized the importance of epidermal features and their relevance in systematic botany. The observations made in leaf epidermal features of the two *Solanum* species are important especially as they help to establish interspecific relationships among the two investigated taxa. For example the similarities in leaf epidermal features showed strong interspecific relationship and thus suggest reasons for the two taxa to belong to the same genus whereas the differences suggest reasons for the two taxa to exist as different species.

Table 1. Epidermal Characteristics of the lower leaf epidermis of *S. macrocarpon and S. nigrum*

CHARACTERS	*S. macrocarpon*	*S. nigrum*
Type of Stomata	Anomocytic	Anomocytic
Stomatal Index	10.14-32.35%	5.70-33.02%
Stomatal frequency	137.40	136.50
Stomatal appearance	Amphistomatic	Amphistomatic
Shape of epidermal cells	Irregular	Pentagonal to rectangular
No of epidermal cells	284.25	347.00

Table 2: Epidermal Characteristics of the Upper leaf epidermis of *S. macrocarpon and S. nigrum*

CHARACTERS	*S. macrocarpon*	*S. nigrum*
Type of Stomata	Anomocytic	Anomocytic
Stomatal Index	6.41-6.80%	3.21-3.64%
Stomatal frequency	39.20	26.10
Stomatal appearance	Amphistomatic	Amphistomatic
Shape of epidermal cells	Irregular	Pentagular rectangular to hexagonal
No of epidermal cells	347	426

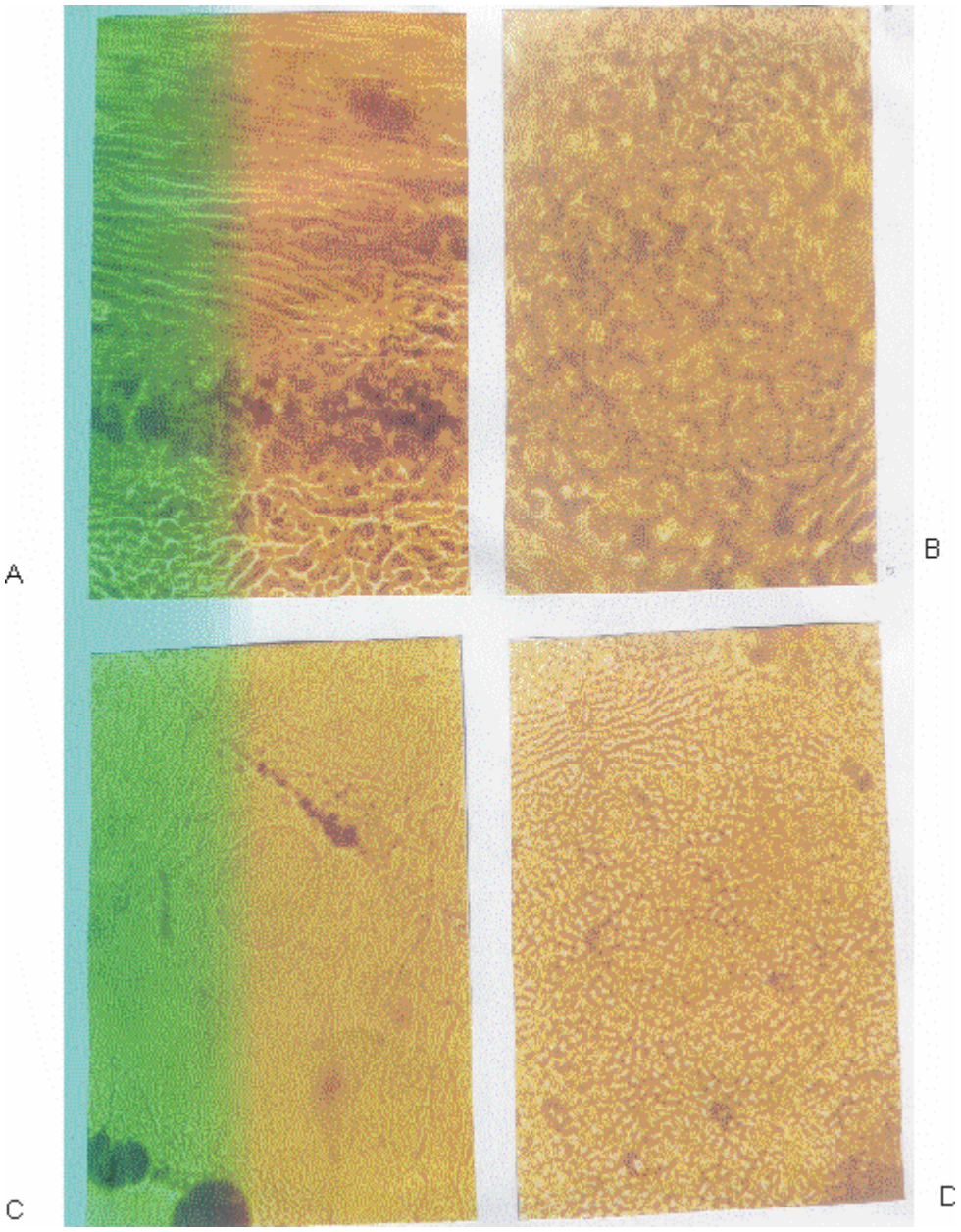

Figure 1 (a-d). Upper and lower leaf epidermis characteristics of the two solanum species studied.

a **Upper leaf epidermal characteristics of S. nigrum**
b **Upper leaf epidermal characteristics of S. macrocarpum**
c **Lower leaf epidermal characteristics of S. nigrum**
d **Lower leaf epidermal of S. macrocarpum**

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Received: July 5, 2007

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Ontogenetic Chain and the Markov Condition in Crop Science

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Abstract: This paper discusses an ontogenetic chain, the specific form of a cause-and-effect system among traits, in relation to crop science. Two approaches to modeling associations in such a system are presented, one based on a so-called sequential approach and another, on the Markov condition. It is shown that usually the former has much stronger biological basis than the latter. [Nature and Science. 2007;5(3):5-8].

Keywords: cause, effect, interpretation, yield components.

The general definition of an ontogenetic chain in relation to plant traits is that the traits that constitute it develop in a certain order during ontogeny, a fact that may result in a particular form of relationships among the traits (Mądry et al., 2005). We may distinguish two main approaches to postulating these relationships ("postulating" because at the very beginning of the investigation we do not know which of the relationships are true and important and which are not, but we may postulate which traits *may be* causes or effects of other traits). The first approach consists in applying what we will hereafter call the all-effect sequential model (the ALL-SEQ model); and the second, in applying the model based on the Markov condition (the MC model), a model that represents the causal chain, quite common in the philosophy of causation. In fact, both of these models are "sequential" because they both take into account the sequence of events in the ontogenetic chain. The name "all-effect sequential model" comes from the fact that the model comprises all the direct and indirect effects that are possible in the ontogenetic chain (see Figure 1); methods that use this model are, for example, sequential yield component analysis (e.g., Eaton and Kyte, 1978; Eaton and MacPherson, 1978) and sequential yield analysis (e.g., Mądry et al., 2005; Kozak et al., 2006).

We are not discussing here the methods of analyzing relationships among the traits in the ontogenetic chain. Our aim is to discuss models that describe these relationships; such a model is to be postulated prior to the analysis, and it may influence its results. (Note that some methods, e.g., path analysis, may be used to study a causal process based on various models; hence the choice of a model matters.) We assume that such postulation is to be done based on the knowledge of a process one aims to study. Therefore, for example, we know that SPAD measurement in the DC 31 stage (SPAD 31; Zadoks et al., 1974) may influence SPAD 49, but the opposite situation (in which SPAD 49 would influence SPAD 31) is impossible (Samborski et al., 2006).

Figure 1 presents the ALL-SEQ model for three traits. In this system the traits that are prior in the chain to other traits may influence them, but may not be influenced by those traits that follow them. A particular, jth trait (X_j) may be, then, determined by all the traits previous to it, that is, X_i for $i = 1, \ldots, j - 1$, and may determine the traits that follow it, that is, X_k for $k = j + 1, \ldots, p$, where p is the number of traits in the ontogenetic chain. Therefore, it is assumed possible that the influence of the jth ($j = 2, \ldots, p - 2$) trait on the last, pth trait (which is often called the dependent trait) may have a direct as well as indirect character, the latter via the traits that follow X_j in the chain (of course, the last but one trait may have only the direct effect on the last one). So, the first trait in the chain may influence all the other traits, not being influenced by any of them, whereas the last trait may be influenced by all other traits, not influencing any of them. This type of causal system is often used to model the associations among traits in an ontogenetic chain in agronomy and crop science; see, for example, Grafius (1969), Rasmusson and Cannell (1970), Thomas et al. (1971), Eaton et al. (1986), McArthur and Eaton (1988), Freeman et al. (1989), Bowen and Kliewer (1990), Akwilin Tarimo (1991), Dofing and Knight (1992), Shamaila et al. (1992), Bos and Spaarnaij (1993), Spaarnaij and Bos (1993), Gołaszewski (1996), Gołaszewski et al. (1998, 2001), Spaner et al. (1996, 2000, 2001), Mądry et al. (2005), Samborski et al. (2006), and many others.

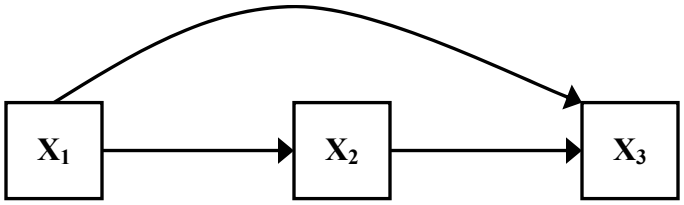

Figure 1. The all-effect sequential (ALL-SEQ) model presenting the associations among the traits in an ontogenetic chain (for three traits).

The modeling based on the MC model is different from that based on the ALL-SEQ model. A description of the Markov condition may be found, for example, in Pearl (2000); its illegible explanation in relation to ecology, in which this model has found many applications (see, e.g., Baker,1989 and Tucker and Anand 2004 and the citations therein), but also plant physiology, was presented by Shipley (2002, sec. 2.7). Under the Markov condition a *j*th ($j > 1$) trait in the ontogenetic chain (note that this paper is concerned with the ontogenetic chain so the Markov condition is discussed only in this context, although it may also be used for other models) is determined *only* by the trait that the *j*th trait follows. Analogously, every trait (except for the last one) in the chain is assumed to be a cause of only one trait: the one it precedes. In this way every trait in the model may be a cause of only one trait as well as an effect of only one trait. The model of this type, for three traits, is presented in Figure 2.

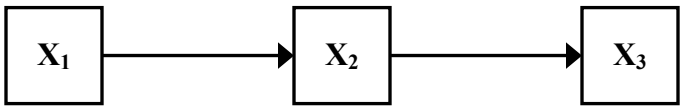

Figure 2. The model based on the Markov condition (the MC model) presenting the associations among traits in an ontogenetic chain (for three traits).

The choice of one of these two models is essential: it is likely that the interpretations based on them will be different (it is likely but not certainly because it may happen that the model in Figure 2 will be true; in such an instance this should be shown also by the analysis of the ALL-SEQ model). Below we compare the two models for one of the most common crop science problems—yield component analysis (Kozak and Mądry, 2006), although what will be said may be linked to many other causal systems in crop science.

Consider the following components of cereal grain yield (GY): number of spikes per unit area (NS), number of grains per spike (NG), and kernel weight (usually presented as 1000-kernel weight, TKW). The order of the traits in the ontogenetic chain is acknowledged to be as follows (e.g., Dofing and Knight 1992): NS, NG, TKW and GY. The MC model assumes in this case that the influence of TKW on GY is direct; the influence of NG on GY is *only* indirect, via TKW; and the influence of NS on GY is *only* indirect, via the path NS → NG → TKW → GY. Thus, the only yield component that directly affects cereal grain yield would be 1000-kernel weight. It is easy to imagine that not too many crop scientists would agree with this statement. Number of spikes per unit area may also have an influence (for example, the direct effect) on cereal grain yield of different character from that along the above-given path (e.g., Rozbicki, 1997). How, then, in the light of the above discussion should we interpret the MC model, in which number of spikes per unit area is *assumed* to affect grain yield *only* indirectly?

In contradiction to the MC model, the ALL-SEQ model assumes that each trait (yield component in our example) *may* (so does not have to) affect the final trait in the chain (grain yield) directly and, for all traits except for the last but one trait, indirectly.

Let us now come back to the example concerned with SPAD measurements. Consider the following ontogenetic chain: SPAD 31, SPAD 49 and winter triticale grain yield. Why should we assume that SPAD 31 has no direct influence on grain yield and the only influence it has on grain yield is indirect, via SPAD 49? Is there any knowledge that would support such a statement? If no, we may simply choose the ALL-SEQ model and try to find which associations (see Figure 1) are and which are not significant and important. Note once more that we do not assume that all associations from the ALL-SEQ model are significant: we assume they are possible.

These simple examples show that the MC model quite often is not correct in crop science. For many, which does not mean that for all, causal processes in crop science that are based on the ontogenetic chain the ALL-SEQ model has much stronger biological basis than the MC model. Of course, this is a researcher's task to decide which model to choose, and it never should be done by convention: the knowledge of the process should provide necessary information.

Nonetheless, looking more generally at the MC model it is easy to notice that this type of approach to analyzing cause-and-effect relationships is quite a simplification of a process represented by the ontogenetic chain. One could say that the assumption that each trait (except for the first one in the ontogenetic chain) has only one cause, that is, the preceding trait, is rather artificial and usually has nothing to do with the biology of the processes. And note that by applying the MC model we are not studying whether or not this is true: we *assume* this. Is there any crop scientist who would agree with the following conclusion: "The only influence that number of kernels per spike has on cereal grain yield is indirect, via 1000-kernel weight, and the only influence that number of spikes per unit area has on cereal grain yield is that via the following path: number of spikes per unit → number of kernels per spike → 1000-kernel weight → grain yield"? A question arises: Is such a simplification of biology appropriate?

In the light of the discussion presented in this paper we may acknowledge that the ALL-SEQ model in crop science is *usually* correct, in contradiction to the model based on the Markov condition, which usually does simplify the biology of crop science processes. This is why Dofing and Knight's (1992) choice of the model of how components influence small grain yield may be acknowledged to be correct. And this is why the choice to use the ALL-SEQ model, not the MC model, in so many analyses (see the papers cited above) may be acknowledged to be correct.

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Received: July 11, 2007

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Opinions and debates

eThekwini Urine Diversion Toilets: A Threat to Groundwater

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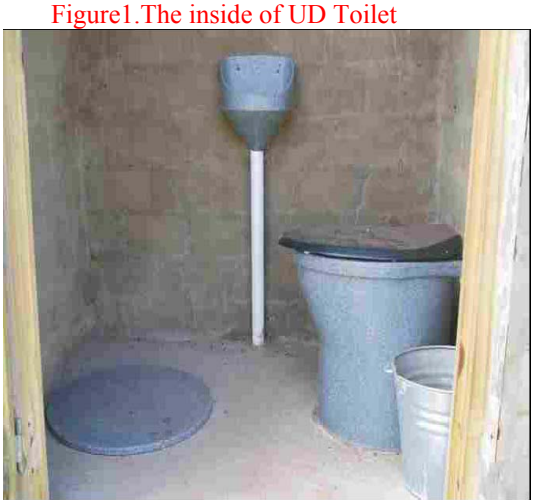
Abstract: eThekwini municipality of South Africa has embarked on the use of urine diversion toilets as a means of providing a basic sanitation to its households. These urine diversion toilets are designed and constructed to be used in a seated position so that the faeces are safely directed into a vault and the urine simply directed into an adjacent soak pit. In this way, urine will induce nitrate contamination into the groundwater system; which if consumed at higher concentrations can be very harmful to humans. Therefore, improvement in urine collection of the eThekwini urine diversion toilets is paramount in order to safeguard groundwater resources from nitrate contamination.
[Nature and Science. 2007;5(3):9-11].

ethekwini's Water and Sanitation Programme in South Africa aims at providing an acceptable and basic level of water and sanitation to households in ethekwini municipality's rural and peri-urban communities by 2010 through the supply of urine diversion toilets and 200-litre yard tanks (WIN-SA,2004). Urine diversion toilet is a form of waterless ecological sanitation designed to separate urine and faeces so that the faecal matter remains dry and rendered disease free for safe handling over time. The ethekwini design uses a two vault system. Once the first vault is full, the pedestal is moved over to the second vault and the first vault is sealed. When the second vault is full, the first vault is emptied and so on (WIN-SA, 2004). The design incorporates a pedestal with two openings; one in front for passing urine and another large one behind for passing faeces, all in a seated position. The faeces go in the vault and the urine is **simply directed to the soak away pit**. Figure 1 below shows the inside of the eThekwini urine diversion toilet.

Figure1.The inside of UD Toilet



In fact, urine-diversion sanitation has been used worldwide for hundreds of years but with careful handling of urine; not merely directing it into the ground .In Yemen, urine is drained away (in urine diversion toilets) and evaporated on the outer face of multistorey buildings to obtain the dry faeces for later use as fuel (Esrey et al., 1998). In Sweden urine has been diverted and used to smear wounds and dry skin (Frode-Kristensen, 1966). Other historic uses of diverted-urine include tanning of hides and production of gunpowder (Stenström, 1996).

In the traditional EcoSan toilet, the system is designed to handle both urine and faeces in combination. In this case, urine helps in the removal of odors due to additional ventilation it causes because of evaporation into the atmosphere through the vent. As a result of natural biological process, the combined human waste gets broken down into dehydrated odorless compost like material. This dry waste is manageable and can be used in the making of compost or disposed off to municipal waste services or used as a source of fuel without danger to groundwater.

Unfortunately, eThekwini urine diversion toilets are not EcoSan toilets; they are best described by Duncan Mara (2006) as urine-diverting alternating twin-vault ventilated improved vault (or VIV) latrines. While urine diversion is good for the performance of this toilet, it can also be a great environmental threat to groundwater quality. In fact, urine has a high potential of inducing nitrate contamination in the groundwater. Historically, the primary sources of nitrate in groundwater have been untreated human sewage from septic tanks and fertilizer applications (Puckett, 1995). The source of nitrate in effluents from septic tanks is urea $\{ (CO(NH_2)_2 \}$ derived from human urine (Layton, 2002). This means urine once released in the soil, urea is introduced which subsequently gets converted to nitrate. Actually urea reacts rapidly with water (in the soil) to form ammonium. This ammonium subsequently undergoes bacterially-mediated oxidation to form nitrate: $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$. Since nitrate is soluble in water, it is eventually carried down to the underlying aquifer by percolating rain, irrigation water or snowmelt. In addition to being soluble in water, nitrate is very stable, and therefore rarely combines with other compounds. Furthermore, it does not bind to soil particles like many contaminants do. This means that nitrate will move with groundwater and can pose problems even kilometers away from a potential source. Consumption or ingestion of groundwater contaminated with nitrate at concentrations greater than the WHO drinking water standard of 45mg/L may cause methemoglobinemia in infants, cancer and other diseases (Downs et al., 1999; Follet, 1989). Basically Nitrate (NO_3^-) is not in itself harmful to human beings and animals. But once it is ingested by the organism, it is converted by bacteria present in the organism, into Nitrite, which subsequently reacts with blood Hemoglobin to form compound called Methemoglobin. This compound reduces the blood's capacity to carry oxygen. The oxygen level then decreases, thus causing a disease called methemoglobinemia also known as "blue baby disease*"* to children under the age of 6, at a period when their digestive system is not mature enough to detoxify this compound. Chronic consumption of high levels of nitrate may also cause other health problems, for example some cancers and teratogenic effects; data are inconclusive, but cause for concern (Kross et al., 1993). According to the EPA (Environmental Protection Agency, U.S.), long-term exposure to water with high nitrate levels can cause diuresis (excessive discharge of urine), increased starchy deposits, and hemorrhaging of the spleen. Consequently, a maximum contaminant level (MCL) of 10 mg/L has been established by the U.S. Environmental Protection Agency for nitrate in drinking water. This is a health-based standard set because of the health risk to infants (Puckett, 1995).

Therefore, urine diversion toilets which direct urine into the ground are potential sources of nitrate contamination of groundwater. This is something that 'EcoSanologists' don't really approve of (Mara, 2006). eThekwini urine diversion toilets could be improved in the method of urine collection so that groundwater which takes decades or even centuries to recover once contaminated may be jealously protected. Above all, diverted urine once properly collected can be put to better uses with less impact on groundwater resources.

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Received: July 27, 2007

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Adoptability of Planted Fallows and Efficacy of Natural Types in Fertility Regeneration of a Typic Paleudult

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ABSTRACT: We investigated the willingness to adopt planted fallows as replacement to the natural fallows among farmers in Owerri Agricultural Zone of Central Southeastern Nigeria, in 2006. Structured interview schedule was used to generate socioeconomic data from respondent farmers. Data were analyzed using percentages and multiple regressions for socioeconomic analysis. Soil samples were also collected from soils under natural fallows but of 5 different fallow lengths. These surface soil samples were analyzed using laboratory techniques for status of soil fertility indices. Resulting data were subjected to Analysis of Variance (ANOVA) and means were separated using the least significant difference (LSD) at 5% level of probability. While analysis of socio-economic data showed that adoptability of fallows had good relationship with education, age and farm size; soil data indicated inability of natural fallow to cope with soil productivity demands at all fallow lengths studied when judged with existing standards. Establishment of planted fallows in demonstration farms of Agricultural Development Programmes (ADPs) of the agroecology, and studies on them may enhance certainty in the prediction of adoptability of these novel techniques of soil fertility regeneration. [Nature and Science. 2007;5(3):12-19]. (ISSN: 1545-0740).

Key word: Degradation, adoption, efficacy, fallows, soil fertility

INTRODUCTION

Over the last couple of decades, conservation agriculture has gained increasing interest in Africa and worldwide, in an attempt to eradicate extreme poverty and hunger; ensure environmental sustainability and develop a global partnership for development (IIRR and ACT, 2005). In response to these problems, some researchers look specifically at soil fertility replenishment systems (CTA, 2002; Place et al., 2005).

Soil fertility restoration strategies have been suggested. The *Gliricidia* and *Leucaena* cropping fallows were recommended since the fallows maintained maize yields at 3.5 tha-^{1}, over six planting seasons without fertilizer (Mafongoya et al., 2001). Yields markedly increased by *Gliricidia manuring* to an average of 1800-2500 kg ha-^{1} (Bohringer and Akinifesi, 2001). In Zimbabwe, farmers apply plant litter from woodlands on very sandy soils (Nyati and Cambell, 1994) while in Malawi, over 1000 farmers used improved fallows, relay cropping and mixed cropping with efficacy of intercropping and closely spaced *Leucaena* hedgerows on soil conservation and maize yield on sloping terrain of Malawi.

In the Central Southeastern Nigeria, there is widespread land degradation due to accelerated soil erosion (Igwe. 2003) resulting to a decline in soil productivity. Increased demographic pressure has led to conflictive land uses and shortened fallow lengths (Onweremadu, 1994) as farmers still hold tenaciously to traditional fallow practices. There is increased deforestation activity and resultant erosion damages of soil resource (Oti, 2007). It is also probable that sociopolitical and anthropogenic activities may have added a negative weight on biophysical factors (Boers, 1990), and this tends to question the capacity of traditional fallow systems to sustain soils for agriculture and non-agricultural uses. Oti (2007) called for an assessment of traditional fallows for the restoration of erosion degraded lands of Southeastern Nigeria and this becomes more expedient with the current campaign for food security. Based on the above, we investigated the efficacy of current traditional bush fallow on owner- managed farms of Central Southeastern Nigeria.

MATERIALS AND MEHTODS

Study area: The study was conducted in Owerri Agricultural Zone in the central Southeastern Nigeria, lying between latitudes $5^{0}15^{1}3.15$"and $5^{0}45^{1}10.21$"N, and longitudes $6^{0}45^{1}8.15$" and $7^{0}30^{1}15.11$"E. The land area of the agricultural zone is about 300 km^{2} and comprises eleven local political units, namely Aboh Mbaise, Ahiazu Mbaise, Ezinihitte Mbaise, Ikeduru, Mbaitolu, Ngor Okpala, Oguta, Ohaji/Egbema, Owerri North, Owerri Municipal and Owerri West. The area is characterized by very high

population density of about 1150 persons per square Kilometer, and this situation is increasing due to rapid urbanization of the area. Soils of the area are formed from coastal plain sands and are classified as *Typic Paleudults* (Onweremadu, 2006). It is a lowland area with humid tropical climate having a rainfall of over 2500 mm and mean annual temperature of 26-29 ^{0}C. Tree plants dominate the vegetation popularly referred to as the rainforest belt of Nigeria. Farming is a dominant socio-economic activity of the study area. Farmers still stick to traditional slash-and-burn system of clearing and soil fertility regeneration is by natural bush fallow where are soils allowed to regain their lost nutrients without intentional input from farmers.

FIELD SAMPLING

Field studies were conducted in 2006, in which three local government areas were purposively chosen based on intensity of farming activities. The three local government areas were Ohaji-Egbema, Owerri West and Ngor Okpala. In each of the three local governments, two towns were randomly selected as follows: Umuagwo and Umuoknne (Ohaji-Egbema), Emeabiam and vu (Owerri West0 and Okpala and Nnorie (Ngor-Okpala). Twenty arable and owner managed farmers were randomly chosen from each town in the local government area. One hundred and twenty arable farmers constituted the sample size for the purpose of this investigation. These farmers are registered with the Agricultural Units of ADP with their respective local government areas.

Structured interview schedule was used to elicit information from the farmers. The structured interview schedule was validated using the content validity technique, which according to Chuta (1992) is used to determine the relevance and suitability of items included in the study. Items contained in the draft interview schedule for the study were validated and thoroughly examined criticized by three lecturers in the Department of Agricultural Extension, University of Nigeria, Nsukka, Nigeria. The final structured interview schedule for the study was certified by the expert opinions of these lecturers. Socioeconomic attributes studied include age, educational status, membership of social organizations and farm size.

Based on personal communication of respondent farmers, five fallow lengths, namely continuous cropping, 3-,5-,10 - and 15 - years fallows were identified and 10 surface soil samples (0-20 cm depth) were collected from each fallow length for laboratory analysis of fertility indicators.

LABORATORY ANALYSIS

Cation exchange Capacity (CEC) was determined by repeated saturation using I M NH_4OA_c followed by washing, distillation and titration (Soil Survey Staff, 1996). Exchangeable basic cations were estimated by inductively coupled plasma atomic emission spectrometer (ICP-AES) (Integra XMO, GBC, Arlington Heights, IL). Available phosphorus was measured by Olsen method as described by Emteryd (1989). Total nitrogen was determined by Kjeldahl digestion with a Kjeltec Auto 1030 System (Tecacor, Hoganas, Sweden). Soil organic carbon was estimated after combustion on a Leco Model 521-275 (Leco Corporation, Svenka AB Upplands, Vasby, Sweden). Soil pH was measured potentiometrically (I:I soil to solution) in water (Thomas, 1996). Base saturation was calculated as the sum of exchangeable basic cations divided by cation exchange capacity, multiplied by 100 percent.

DATA ANALYSIS

Descriptive statistical tools were used in analyzing collected data. Analysis of variance (ANOVA) was used to determine variation among soil data and means separated using least significant difference (LSD) while willingness to adopt planted fallow technology (Dependent variable) was regressed to selected socio-economic characteristics as independent variables.

The multiple regression model used is shown as follows: $Y= a+ b_1X_1+b_2X_2+b_3X_3+b_4X_4+e...$ 1
where Y = Willingness to adopt planted fallow

a = intercept

B_1b_4 = regression coefficients

X_1 = age

X_2 = education

X_3 = membership of social organization

X_4 = farm size

e = error term.

Results

The socioeconomic characteristics of respondent farmers are shown in Table 1, indicating that most farmers in the study site are of youthful age bracket (70%). Again, secondary and primary education dominated the educational status of these respondent farmers while fewer farmers engaged in more than four social organizations. Farm sizes were generally low (40%) to every low (32%). These results contrasted with the findings of Nwuzor (2007) in Abakaliki, another part of the same agroecology where farmers were older and less educated. But, Agwu and Chukwu (2006) reported that age of most farmers in Aninri Enugu State Nigeria lies within 20-39 years, a result resembling the trend in this study.

Natural bush fallow, farmyard manure and mulching dominated the existing soil conservation practices in the area when compared with the use of diversion ditches and terracing (Table 2). This is consistent with the findings of Odii (2002) that bush fallowing dominated soil conservation against soil erosion in this agroecology. Although, there exists sources of modern soil conservation practices in the area (Table 3), bush fallowing is allowed to naturally check environmental influences on soil resource and maintain soil fertility. However, agricultural extension services occupy 38% of the sources of information on soil conservation, followed by education and mass media, yet their influences have not changed the tenacity to which farmers hold to traditional bush fallow practice. This is because about 56% of the respondent farmers were not aware of the use of planted fallow technology (Table 4). Among the population with the knowledge of planted fallow technology, 55% are willing to adopt planted fallow technology (Table 5), indicating that a good proportion (45%) of the respondents are currently unwilling to accept and adopt the fertility regeneration and soil conservation technology.

Statistical data on the relationship between willingness to adopt planted fallow technology and socioeconomic attributes (Table 6) indicated that age, education and farm size were significantly (P=0.05) related to adoption. While education had a significant positive correlation with willingness to adopt, Korie et al (2006) reported a significant negative relationship between education and adoption of land tenure system, suggesting that modern farming agriculture may cease to adopt traditional land tenure system.

Status of soil fertility indicators in five natural fallows of the study area is shown in Table 7. Using existing standard (SPDC, 2003), values of cation exchange capacity, soil organic carbon, total nitrogen and available phosphorus were very low to moderate, and soils were strongly acidic irrespective of fallow length in natural fallows. These were significant differences among fallow lengths in measured soil attributes (Table 7).

Table 1: Distribution of respondents according to socio-economic characteristics (n=120)

Attribute	Percentage
Age (Years)	
21-30	25.0
31-40	45.0
41-50	20.0
51 and above	10.0
Education	
No formal education	6.3
Primary education	30.2
Secondary education	51.0
Tertiary education	12.5
Membership of social organizations	
1-2	51.0
3-4	36.1
5-6	12.9
Farm size (Hectares)	
1.0	32.0
1.1-2.0	40.0
2.1-3.0	18.0
7-3.0	10.0

(Source: Field Survey, 2006).

Table 2: Distribution of existing soil conservation practices

Practice	Percentage
Natural Fallowing	68
Mulching	10
Farmyard manure	12
Terrace	4
Diversion ditch	6

Table 3: Source of modern soil conservation knowledge (n=120)

Source	Percentage
Agricultural Extension Service	38
Education	24
Mass media	20
Farmers organization	18

Table 4: Knowledge of planted fallow technology (n=120)

Awareness level	Percentage
Unaware	56
Aware	44

Table 5: Willingness of respondent farmers to adopt planted fallow technology (n=120)

Attribute	Percentage
Highly unwilling	20
Slightly unwilling	25
Willing	30
Highly willing	25

Table 6: Multiple regression analysis on the relationship between willingness to adopt planted fallow and socioeconomic characteristics (n=120)

Independent Variable	Coefficient	SE	T-value	F- ratio	R^2 (adj)
Constant	62.15	0.92	16.60*	3.02	0.36
Age	-9.36	0.08	-7.32*		
Education	13.24	0.09	8.15**		
Membership of Social organization	6.21	0.11	1.63 ^{NS}		
Farm size	-11.23	0.05	-5.22*		

Table 7: Soil fertility indicators in natural fallows of the study cite

Length of Fallow (Water) (Years)	pH	CEC (meq/100g Soil)	BS (%)	SOC (%)	TN (%)	Avail.P (ppm)	
Continuous Cropping	2.8	29.0	0.5	0.08	5.8	3.8	
3	3.9	36.2	0.6	0.10	6.2	4.0	
5	5.8	39.8	0.8	0.14	9.6	4.1	
10	11.3	42.3	1.2	0.18	15.2	4.6	
15	14.7	47.2	1.4	0.20	18.6	5.0	
$LSD_{(0.05)}$	0.8	0.6	0.3	0.08	0.3	0.7	

CEC = cation exchange capacity, BS = base saturation,
SOC = soil organic carbon TN = total nitrogen, Avail. P = available phosphorus, LSD = least significant difference.

DISCUSSION

The implication of the predominance of youthful age (45%) in Table 1 is that agricultural extension services should focus on such population moreso, where 51% of them have attained secondary education. Ozor and Madukwe (2005) suggested the use of Youth Farmers Clubs (YFCs) for such target population. However, we suggest that Agroforesty Clubs (AFCs) be mounted in secondary schools where experimental plots on planted fallows could be used for demonstration purpose by Agricultural Science Teachers (ASTs). In Southeastern Nigeria, educational level has been found to be one of the most closely related variables to adoption of improved farm practices (Mathews-Njoku and Asiabaka, 2003). The intervention of government and non-government bodies in encouraging the population of this educated youthful and emerging farmers is so desirable to forestall the migration of this category of human capital which were trained by urban settlement in South African agriculture (Ortmann and Machethe, 2003).

A good number of the respondent farmers (51%) had only 1-2 social organizations, which is an indicator of their rickety economic foundation as farmers may likely belong to more social organizations as income increases given the African cultural peculiarity. It is of note that social organization serves as forum through which farmers could exchange ideas and learn about new farm practices (Onu, 1991). However, social organization vary in the study area comprising agro-based groups, purely entertainment social groups, age-grade organizations, and spiritual groups but this classification was beyond the scope of the study.

About 72% of the respondent farmers population had less than 2.0 hectares of farmland, suggesting higher possibility of adoption of planted fallows in the area as a way of quickly rejuvenating soil fertility in a very short temporal specification. The more the land mass, the greater the propensity to hold to traditional bush fallow since farmers can afford to allow their farmlands rest for years without cultivation.

One would have expected a diminishing prominence of natural bush fallow in the area given high percentage of small-sized farms, but it was to the contrary (Table 2). It implies from the foregoing that respondents are left to regenerate their fertility or that they cultivated under shortened fallow lengths in line with the findings of Onweremadu (1994). Despite the leading position of agricultural extension service as source soil conservation knowledge (Table 3) it has not succeeded in attracting a good number of farmers to adopt planted fallow technology which has been a common practice in other African Countries (CTA, 2002). This is worst still with 56% of the respondents indicating ignorance of the technology (Table 4). It means that part of the Millennium Development Goals should focus great attention on such innovations as planted fallow technology since it takes the traditional soil fertility restorative technology longer time. Efforts in this direction must remember the inculcation of indigenous knowledge peculiar to the locality (Onweremadu et al., 2007).

Field studies also showed that many respondent farmers (55%) are willing to adopt planted fallow technology, implying that a lot of extension services to convert 25% of the "slightly unwilling" population

to "willing" status (Table 5), willingness to adopt could be a direct response to low productivity of farmlands in the study area. Although the study did not evaluate soil productivity vis-à-vis crop performance, it was demonstrated by farmers during personal communication at different sampling points. The propensity to adopt planted fallow technology as a replacement to the traditional bush fallow system was regressed with studied socio-economic attributes and results indicated a very good positive relationship between adoption and education (T-Value = 8.15**), suggesting that adoption increase with enlightenment as an educated farmer can understand technologies which appear complex to the uneducated (Egbule and Mathews-Njoku 2002). Farm size had a significant negative relationship (T-Value = 5.22*) with adoption of planted fallows, implying that as farm size increases propensity to adopt decreases. The traditional fallow period is promoted by large farmlands amidst low farmer populations. But, the population density of the study area is so high amidst traditional land tenure system which promotes fragmentation and shortening of rest period. In the same manner, age had a significant negative relationship (T-value = - 7.32*) with willingness to adopt, showing that older farmer respondents were less willing to adopt this technology, possibly due to cultural attachment and disposition (Onweremadu and Mathews-Njoku, 2007) while younger population would want to increase their "pocket money" by any promising economic venture (Okoro, 1991).

Soil analytical data (Table 7) exhibits the relative productivity of soils at different fallow periods under natural bush fallow system, with data confirming the inability of popularly adopted system of soil fertility regeneration. In the context of increasing demographic pressure amidst rising and conflicting demands for farmland resources, soil resource is subjected to several forms of degradation. This is worst for continuously cultivated soils in a low-input farming system. Although Oti (2007) suggested the use of strategies which improve soil structure, soil organic matter levels, plant available water, essential nutrient reserves, and diversity of soil biodiversity to sustain traditional fallows, social understanding and applicability of the strategies may be difficult. The recalcitrance of farmers to adopting modern soil conservation strategies is worrisome since Zake (1993) reported failure of inorganic fertilizers in restoring yield levels in degraded Ultisols of Uganda, and soils of the site are mainly Ultsols whose landscape has been ravaged by soil erosion (Onweremadu, 2006). Efficacy of improved fallows for oil fertility replenishment using *Sesbania, Glicicidia* and *Tephrosia* has been reported in Kenya, Uganda, Zambia, Malawi and Zimbabwe (CTA, 2002), and could be used in soils of the study area. Evaluation of these fertility enhancing plants in combination with arable crops may be necessary for appropriate recommendations. This is because seed supply and viability may be unreliable hence a disincentive to farmers in such marginal environment of transitional economics. It may be necessary to resort to farmer decision-making models and econometric analyses (Benin et al., 2004; Gauchan, 2004), which consist of channels through which farmers acquire information and interact with the farmers who are practicing the technology in their farms.

CONCLUSION

The study revealed that majority of respondent farmers belong to youthful age bracket and with secondary education. Farmers belong to few numbers of social organizations and are mainly low income farmers based on the small farm holdings. Result also showed that natural fallows are still dominant, irrespective of their knowledge of modern soil conservation practices. However, majority of the farmers are unaware of the planted fallow technology although a good number of them are willing to adopt it. We also found that socio-economic characteristics such as education, age and farm size influenced willingness of respondent farmers to adopt, suggesting future use of these attributes in modeling adoption of improved and planted fallows in the study site, and this will certainly enhance precision farming in the study area.

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Received: 8/1/2007

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New Explanations to the Accelerating Expansion of Our Universe: It Might Be Caused From the Collision between Two Universal Black Holes in Their Early Years

---- Part 5 of "New Concepts to Big Bang and Black Holes" [1][2]----

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Abstract: In 1998, two groups led by Professor Saul Perlmutter of Lawrence Berkeley National Laboratory 50-232 University of California and Brain Schmidt of Australia National University individually discovered the accelerating expansion of our universe (AEOU) through the observations to the bursts of supernovas Ia, they pointed out, that the remote galaxies are accelerating away from us.[3] Lots scientists regarded the existence of mystical dark energy (DE) of exclusive force or negative energy as the origin of AEOU, some of them are making their great efforts to find out dark energy for winning Nobel prize. Especially, our universe was born from Big Bang about 13.7 billion years ago,[3] no dark energy appeared along with the birth of our universe, it just cropped out about 9 billion years ago.[3] What is dark energy? Nobody knows it at present. Physics Professor of China Science and Technology University, Li Miao jokingly said: "How many specialists of dark energy there are, how many kinds of dark energy may be imagined out."[3] Can AEOU be explained only by dark energy of exclusive force or negative energy? According to theories and the innate natures of black holes (BH), the expansion of a black hole can be caused by swallowing in energy-matters from its outside and by collision with other black holes (BH). The more energy-matters could be swallowed in, the faster expand a black hole (BH) would [see formulas (6b), (6c) below]. In this article, the accelerating expansion of our universe (AEOU) will be explained with the collisions between our universal black hole (UBH) and another one in their early years. Though the demonstrations in this article may be relatively simple; but they are more reasonable than the demonstrations of all current theories to AEOU. [Nature and Science. 2007;5(3):20-29]. (ISSN: 1545-0740).

Key Words: dark energy, dark energy of excusive force, dark energy of negative energy, accelerating expansion of our universe, collision of two black holes, multi-universes, space expansion exceeding light speed, acceleration of the universe

I. The accelerating expansion of our universe has proved the real existence of multi-universes.

The recent observations indicate that, the so-called dark energy did not simultaneously appear with the birth of our universe; it cropped out about 5 billion years later. It clearly shows that, the dark energy surely came from outside of our universe, i.e. from other universes. It is the hard evidence to show the existence of multi-universes. In addition, "Recently, many super-massive BHs (its mass $M_b \approx 10^9 M_\theta$, M_θ –- our sun mass) were discovered in our universal space. According to calculation, its average density is about $\rho_s \approx 0.0183 g/cm^3$."---quoted from paragraph 15 in <New Concepts to Big Bang and Black Holes>.[2] In such super-massive BHs, there would certainly be many stars with its planets. Moreover, there would still be too many energy-matters outside those super-massive BHs. Thus, they could continuously grow up bigger and bigger with swallowing in outside energy-matters. Several billion years later from now on, the intelligent living beings might appear on the planets in some super-massive BHs, they could have no way to know the worlds outside of their BH. It is said, even in our same universe, if there were the intelligent living beings in the different BHs, they could have no way to make any contact each others, because **every black hole (BH) is a completely independent kingdom.** Fortunately, our solar system does not locate in the super-

massive BH of our galactic center, otherwise, our mankind may have no way to know the whole galaxy, let alone know our whole universe. Therefore, the relationships between those super-massive BHs in the different galaxies of our universe are just the same conditions between our universe and other universes outside of ours, because **our universe has been a real gigantic universal black hole** (UBH).[1][2] Above-mentioned super-massive BHs in the different galaxies could grow up after swallowing in energy-matters outside or even collide with other BHs, just as our universe would expand its volume by swallowing in energy-matters outside or colliding with another UBH outside of our universe.

II. The proposal of dark energy, any new theories to the explanations of accelerating expansion of our universe must accord with the Flatness and the current precise observational value of ($\Omega = 1.02 \pm 0.02$) of our universe, the dark energy of exclusive force might become a specter unable to be found.

Einstein's Field Equation of General Theory of Relativity (GTR) as below,

$$G\mu\nu = 8\pi G\, T\mu\nu + \Lambda g\mu\nu \quad [4] \qquad (1)$$

$G\mu\nu$ is Einstein tensor to describe the geometrical character of time-space; $T\mu\nu$ is energy-momentum tensor of matter field; $\Lambda g\mu\nu$ is a cosmological item, in which Λ is so-called cosmological constant. $\Lambda g\mu\nu$ would have exclusive force, which was added later by Einstein for keeping our universal balance between the gravitational forces and exclusive forces.[4] For convenient analyses, $T\mu\nu$ can be divided into three items below.

$$\text{Let} \quad T\mu\nu = T^1\mu\nu + T^2\mu\nu + T^3\mu\nu \qquad (2)$$

According to the recent precise observations and theoretical calculations, $T^1\mu\nu \approx 4\%T\mu\nu$,[3] $T^1\mu\nu$ delegate an item of general visible matters, such as stars, interstellar mediums. According to the observations and theoretical calculations to distributions of rotary speed in many galaxies, $T^2\mu\nu \approx 22\%T\mu\nu$,[3] i.e. $T^2\mu\nu \approx (5 \sim 6)\, T^1\mu\nu$. $T^2\mu\nu$ is an item of dark matters to delegate the invisible matters of gravitational force. $T^3\mu\nu \approx 74\%\ T\mu\nu$,[3] it is so-called dark energy other than $(T^1\mu\nu + T^2\mu\nu)$. The amounts of dark energy with $(T^1\mu\nu + T^2\mu\nu)$ together must maintain Flatness and $(\Omega \rightarrow 1)$ of our universe. However, the predicts of Inflationary Cosmology which was proposed by Guth and Linde, and theoretical researches of cosmological dynamics all required Flatness, Evenness and $\Omega = \rho_r / \rho_o \approx 1$ of our universe, i.e. required that our universal real density ρ_r must extremely approach to our universal critical density ρ_o. Recently, various precise observations have confirmed the correctness of above theory and concepts, i.e. the best observational value of $\Omega = 1.02 \pm 0.02$.[4] Of course, here the required dark energy must have positive energy or gravitational force.

Now, for explaining the newly discovered accelerating expansion of our universe through the observations to the bursts of remote supernovas Ia, many scientists proposed some new theories, they merged above $(T^3\mu\nu + \Lambda g\mu\nu)$ together into one thing -- $\Lambda g\mu\nu$, they considered that $\Lambda g\mu\nu$ included $(T^3\mu\nu = 74\%T\mu\nu)$ as unknown and secret dark energy must have exclusive forces or negative energy. A famous delegate of new theories is quantum field theory (QFT), in which $(T^1\mu + T^2\mu\nu = 0)$ are considered as the vacuum state or the state of the lowest energy or the basic state of quantum field,[4] i.e. zero point energy of microcosm. However, the macro energy-matters i.e. general matters $(T^1\mu + T^2\mu\nu \neq 0)$ of our universe are considered as a excited state of quantum field. Observations to the vacuum state of our universe may very closely accord with $(T^1\mu\nu + T^2\mu\nu) = 0$, thus, $\Lambda g\mu\nu$ is just regarded as vacuum energy, i.e. dark energy of exclusive force to include $T^3\mu\nu$. Unfortunately, **the calculated value of $\Lambda g\mu\nu$ according to QFT is even equal to more than 10^{120} times of the really observational value of $\Lambda g\mu\nu$ in vacuum.** For this reason, QFT has met the greatest trouble to solve Einstein's field equation. Obviously, so much negative energy $\Lambda g\mu\nu$ calculated out by QFT would have no way to maintain Flatness of our universe and identity between the tensor of $G\mu\nu$ in Einstein field equation and the real observational values. QFT seemingly regarded vacuum energy as "the unlimited free lunch". How much vacuum energy could deposit at any place in our universe and be taken out? Why could the negative energy come from vacuum not annihilate with positive energy-matters in our universe? How could guarantee the real Flatness of our universe with 74% dark energy of negative energy $\Lambda g\mu\nu$? Solving above problems may hardly not violate the natural cardinal principle----the law of causality. It can be seen, **any new theories included QFT to the explanations of**

accelerating expansion of our universe must not violate the Flatness of our universe and the recently precise observational value of ($\Omega = 1.02 \pm 0.02$).

In reality, some scientists and some observations did not support the existence of "secret dark energy" and "dark energy of negative energy".

Lioto, scientist of Italy National Institute of Nucleon Physics said: "the accelerating expansion of our universe doesn't need the mystical dark energy, it's just the neglected expansive effect of Big Bang."[5]

Scientists of XMM Newton Astronomical Telescope of Europe Space Bureau observed that the proportions of blazing gases had almost no difference between very old and young clusters of galaxies. It is a better evidence to have showed the non-existence of dark energy[6] and to accord with theoretical demands. Surely, the current total amount of $(T^1\mu\nu + T^2\mu\nu)$ is too little to maintain Flatness of our universe and hardly to let our universal real density ρ_r extremely approach to our universal critical density ρ_o. Therefore, $T^3\mu\nu / T\mu\nu \approx 74\%$ must be needed to have positive energy. However, $T^3\mu\nu$ should be an item of dark energy to delegate those not observed and invisible positive energy in our universe.[3][4]

On January, 8, 2007, an America science research group declared that, through effort in several years, they had firstly drawn up the three dimension map of dark matters in our universe. They pointed out that, in our universe, about 1/6 matters are visible matters, more than 80% matters of the rest are dark matters.[7] They really negated the existence of any dark energy.

Modern traditional cosmology generally merged the cosmological item into energy-momentum tensor of matter field, it is about equal to introduce an energy-momentum distribution of an energy density: i.e. $\rho\Lambda = \Lambda/8\pi G$, pressure $p\Lambda = -\Lambda/8\pi G$.[4] **In reality, modern traditional cosmology from introducing items of $\rho\Lambda$ and $p\Lambda$ had really regarded the exclusive forces of heat pressure as the antagonist of gravitational forces in our universe. Thus, the dark energy of exclusive force is not required in modern cosmology.**

III. The expansive laws of BHs after swallowing in energy-matters outside or after the collision between two BHs.

According to Schwarzchild's special solution to the equation of GTR, the necessary condition for the existence of any real gravitational black hole (RGBH) or so-called Schwarzchild's BH (i.e. no charges, no rotating and spherical symmetry) is:

$$\mathbf{R_b = 2GM_b/C^2, \ or \ R_bC^2/2G = M_b} \quad [9][2] \qquad \mathbf{(3)}$$

M_b-- the total mass of BH, R_b--the Schwarzchild's radius of BH, C—light speed, M_θ —sun mass, G—gravitational constant,

A. when a BH swallows in energy-matters outside,

$$M_b = 4\pi\rho_b R_b^3/3 \qquad (4)$$

From formulas (3) and (4),

$$3C^6 = 32\pi G^3 \rho_b M_b^2 \qquad (5)$$

$$dR_b = (2G/C^2)dM_b \qquad (6)$$

$$dR_b/dt = (2G/C^2)\, dM_b/dt \qquad (6a)$$

Formulas (3), (4), (5) and (6) indicate that, when M_b increases 10 times due to swallowing in energy-matters outside, its density ρ_b would lower 100 times, and R_b equally increases 10 times.

The relative expansive speed V_b of Event Horizon of any BH is: $V_b = 2dR/dt$, so,

$$V_b = (4G/C^2)\, dM_b/dt \le 2C \qquad (6b)$$

In case $dR_b/dt = C$, and $dt = 1$ second, then $dM_b/dt = 2\times10^{38}$g/sec, it is almost equal to swallow in 10^5 solar system in 1 second. $V_b = 2C$ may be the greatest limit of swallowing in energy-matters outside for a BH.

The expansive acceleration a_b of the Event Horizon of any BH is: $a_b = dV_b/dt$, hence,

$$\mathbf{a_b = (4G/C^2)d^2M_b/dt^2} \qquad \mathbf{(6c)}$$

Formula (6c) expresses that, **the accelerating or decelerating expansion a_b of the Event Horizon of a BH is directly proportional to the increasing or decreasing speed of energy-matters to be swallowed**

in by that BH. Then, its swallowing in energy-matters outside is the normal functions of any BHs. From formulas (3) and (6),

$R_b + dR_b = (2G/C^2)(M_b + dM_b)$ (6d)

B. According to formula (3), **if two BHs of M_{b1} and M_{b2} had collided**, R_{b1} and R_{b2} are their respective Schwarzchild's radius, then, $R_{b1}C^2/2G = M_{b1}$, and $R_{b2}C^2/2G = M_{b2}$, as a result,

$$\mathbf{M_{b1} + M_{b2} = (R_{b1}+R_{b2})\, C^2/2G} \quad (7)$$

A new BH would be formed after collision, its mass $M_{bn} = M_{b1} + M_{b2}$, its Schwarzchild's radius $R_{bn} = (R_{b1}+R_{b2})$.

Conclusion: From formulas (6d) and (7), once a BH had formed, no matter whether it increased or decreased energy-matters or even collision with another BH, it would be a BH forever before its disappearance with becoming the minimum gravitational black holes (MGBHs) of 10^{-5}g.[1][2]

IV. Our universe has been a real universal black hole (UBH).

For explaining the characters of our universe as a real UBH, two more accurate observational values about our universe will be adopted to do some calculations below (a). The current age A_u of our universe from Big Bang to the present is: **$A_u = 13.7\times10^9$yrs**.[3] (b**). Hubble's constant $H_o = (0.73\pm0.05)\times100$kms^{-1}Mpc^{-1}** [4]. If above two values are more reliable, the results are as follows. (a). If our galaxy locates in a gigantic universe enough, the current visible radius R_{uv} of our universe is: **$R_{uv} = C\times A_u = 1.3\times10^{28}$cm,** it is said, the farthest stars, which may be observed, is about 1.3×10^{28}cm away from us, it just is the distance of light travel in the universal age A_u. The visible Event Horizon of our universe is equal to $2R_{uv}$. (b). The real observed density **ρ_r of our universe is: $\rho_r = 3\, H_o^2/(8\pi G) \approx 10^{-29}$g/cm^3**.

A. Now according to the observed real density $\rho_r \approx 10^{-29}$g/cm^3, our UBH (M_{ub}) can be calculated from laws of BH. Let M_{ub} are the total energy-matters of our real UBH, R_{ub} is its real Schwarzchild's radius. From formulas (3) $R_{ub}C^2/2G = M_{ub}$, and (4) $M_{ub} = 4\,\pi\,\rho_r\,R_{ub}^{\,3}/3$, and $\rho_r \approx 10^{-29}$g/cm^3, then, **our UBH is formed by: $M_{ub} = 8.5\times10^{55}$g, $R_{ub} = 1.265\times10^{28}$cm, $\rho_r \approx 10^{-29}$g/cm^3.**

B. The definite evidences verify that, our universe (M_{ub}) is a real UBH. If our universe is a real gigantic UBH, it was only originated from small BH or from the mergence of many BHs. So, it should be formed by numbers N_{ub1} of original BHs (i.e. MGBHs its $M_b \approx 10^{-5}$g, $R_b \approx 1.5\times10^{-33}$cm, $T_b \approx 0.65\times10^{32}$K, see reference [1]) at the birth from Big Bang, so, $N_{ub1} = M_{ub}/\text{MGBH} = 8.5\times10^{55}/10^{-5} = 8.5\times10^{60}$. At the same time, from formula (7), $N_{ub2} = R_{ub}/R_b = 1.265\times10^{28}\text{cm}/1.5\times10^{-33}\text{cm} = 8.43\times10^{60}$. **Due to $N_{ub1} = N_{ub2}$,** then, **it is a reliable evidence to show that our universe is a real gigantic UBH.**

C. **The Flatness and ($\Omega = \rho_r / \rho_o = 1$) are the innate natures of our UBH**: according to Hubble's law, in our universe, the relative expansive speed V_p of any point P, which has a distance R_p to a certain spherical center, is equal to,

$V_p = H_o R_p$ (8)

From formula (3) and (4), on the Event Horizon of our UBH, when R_p prolongs to equal to R_{ub}, so, $V_p = C$, then,

$H_o^{\,2} = 8\pi G\,\rho_o/3$ (9)

Since our universe has been a real UBH, it must be a close spherical body; so, ρ_o is the critical density of our UBH and is a sole value only decided by M_{ub} or R_{ub} from formulas (3) and (4).[2] However, the real density ρ_r is originated from the same observed H_o, i.e. $H_o^{\,2} = 8\pi G\,\rho_r/3$. Certainly, ρ_r should be completely equal to ρ_o in formula (9), **because ρ_o and ρ_r are all come from the same H_o.** So, **($\Omega = \rho_r/\rho_o = 1$) is the innate nature of our UBH. Thus, $\Omega = \rho_r/\rho_o = 1$ should conversely verify that our universe is a real UBH.**

D. Now owing to $R_{ub} < R_{uv}$ ($R_{ub} = 1.265\times10^{28}$cm, $R_{uv} = C\times A_u = 1.3\times10^{28}$cm), the farthest real boundary which can be observed by mankind is only the Event Horizon R_{ub} of our UBH, but not the Event Horizon R_{uv} of virtual M_{uv}.

If ρ_{ov} is defined by some theories other than the theory of BH as the critical density of our universe, strictly speaking, formula (3) cannot be adopted to determine ρ_{ov}, because the amounts of the real current M_{uv} outside our UBH can't be known. However, in modern cosmology, ρ_{ov} is just a presumed values, and calculated out on the assumption that M_{uv} and R_{uv} accord with formula (3). Thus**, M_{uv}, R_{uv} and ρ_{ov} would be artificially formed a new virtual UBH (M_{uv})** bigger than our current UBH with a definite $R_{uv} = C \times A_u$. That virtual UBH would be: $R_{uv} = 1.3\times10^{28}$cm, $M_{uv} = 8.77\times10^{55}$g, $\rho_{ov} = 0.95\times10^{-29}$g /cm^3. Therefore, **the so-called Ω has really become to $\Omega = \rho_r/\rho_{ov} = 10^{-29}\text{g}/0.95\times10^{-29} = 1.05$. However, ρ_r/ρ_{ov} is a true proportion of two real densities of two UBHs, but not a proportion between real density and critical density of the same universe. Only owing to $R_{ub} \approx R_{uv}$, $M_{ub} \approx M_{uv}$, $\Omega = \rho_r/\rho_{ov} \approx 1$** at present, **it had been wrongly applied by most cosmologists as a discriminant to judge the future destiny of our universe: close universe or open universe. Since our universe is a real UBH as above demonstrations, it is a close universe. There is no significance to define Ω for any BH included our UBH.**

Therefore, our current visible universe is equal to our UBH-- M_{ub}, but not the virtual UBH-- M_{uv}.

If there'll be still much energy-matters enough outside our UBH (M_{ub}), after n years, M_{ub} will enlarge to the present virtual M_{uv}. $n = (R_{uv} - R_{ub})/C = (1.3\times10^{28} - 1.265\times10^{28})/3\times10^{10} = 3.7\times10^8$yrs. However, at that time, the age A_u of our universe won't be 13.7×10^9 yrs, but equal to $A_u + n = 14.07\times10^9$yrs.

E. The Event Horizon of our UBH, $2R_{ub} \leq (2R_{uv} = 2C\times A_u)$. It shows that, the relative expansive speed V_{ub} of the Event Horizon of our UBH has exceeded light speed C on average (i.e. $V_{ub} \approx 2C$) all the way from Big Bang to the present.

F. The calculated age of our universe t_o: According to the old formula, $t_o = 1/H_o = 1/(0.73\pm0.05)\times100\text{kms}^{-1}\text{Mpc}^{-1} \approx 13.3\times10^9$ yrs, but according to the revised General Theory of Relativity (GTR), $t_o \approx 2/3\times1/H_o \approx 9\times10^9$yrs.

V. The accelerating expansion of our universe (AEOU) should be caused from collision between two UBHs in the early years.

From above demonstration, **since our visible universe is our UBH—M_{ub}, but not M_{uv}, thus, AEOU = AEOUBH (accelerating expansion of our UBH).**

The accelerating expansion of our universe (AEOU) was observed and demonstrated through the observations and calculations to the bursts of remote supernovas Ia. The AEOU happened about after 5×10^9yrs of the birth of our universe or about 9×10^9yrs ago. In this article below, the AEOU will be explained and demonstrated by the collisions between two universal black holes (UBHs).

Assuming that, **about 9×10^9yrs ago, our smaller UBH M_{ub1} collided with or dropped in another greater UBH M_{ub2},** what had happened since then? Of course, such collision was a long-term process.

Suppose M_{ub1} was the total mass of our smaller UBH, $R_{ub1}$was its Schwarzchild's radius, N_{o1} was numbers of MGBHs ($M_b \approx 10^{-5}$g, $R_b \approx 1.5\times10^{-33}$cm, $T_b \approx 0.65\times10^{32}$K) to form M_{ub1}.

Suppose M_{ub2} was the total mass of the greater UBH, R_{ub2} was its Schwarzchild's radius.

The conditions after collision between M_{ub1} and M_{ub2} are analyzed as below.

A. Once our smaller M_{ub1} dropped in the greater M_{ub2} about 9×10^9yrs ago**,** from formula (7), so, $M_{ub1} + M_{ub2} = (R_{ub1} + R_{ub2})\,C^2/2G$, it is said, due to capturing M_{ub1}, M_{ub2} had to increase its Schwarzchild's radius to $(R_{ub2} + R_{ub1})$. Thus, M_{ub2} in Δt times got the expansive speed of its Event Horizon V_{ub22}, $V_{ub22} = R_{ub1}/\Delta t$. If the Event Horizon of M_{ub2} did not fully expand to R_{ub2} before collision, M_{ub2} should have a speed V_{ub21} of repercussions of initial Inflation. If there might be energy-matters outside swallowed in by M_{ub2}, it would get other expansive speed V_{ub23}. Then, **the total expansive speed of M_{ub2}'s Event Horizon V_{ub2} of M_{ub2} is: $V_{ub2} = (V_{ub21} + V_{ub22} + V_{ub23})$.**

B. Let's look back to our original M_{ub1}, once the original M_{ub1} dropped into M_{ub2} about 9×10^9yrs ago, it could swallow in energy-matters from M_{ub2}, from formula (7), **M_{ub} is our present UBH turned from M_{ub1}**, so, $M_{ub} = M_{ub1} + \Delta M_{ub12} + \Delta M_{ub13} = (R_{ub1} + \Delta R_{ub12} + \Delta R_{ub13})\,C^2/2G = R_{ub}C^2/2G$. If M_{ub1}did not fully expand to R_{ub1} before collision, M_{ub1}should have a speed of its Event Horizon V_{ub11} of repercussions of initial Inflation. Under the general condition, M_{ub1} could only swallow in ΔM_{ub12} from M_{ub2}, it would lead R_{ub1} more expand to ΔR_{ub12} and get expansive speed $V_{ub12} = \Delta R_{ub12}/\Delta t$. However, **due to that M_{ub2} had**

the total expansive speed V_{ub2} = (V_{ub21} +V_{ub22} +V_{ub23}), M_{ub2} could let M_{ub1} cause an additional space expansion ΔR_{ub13}, and led M_{ub1} swallow in more energy-matters ΔM_{ub13}. So, M_{ub1} could get an additional space expansive speed $V_{ub13}= \Delta R_{b13}/\Delta t$. Thus**, the total expansive speed of M_{ub1}**'s **Event Horizon V_{ub1} of M_{ub1} is: $V_{ub1}= V_{ub11}+ V_{ub12}+ V_{ub13}$.**

Conclusion:

Due to that our smaller UBH M_{ub1} dropped in another greater UBH M_{ub2}, and M_{ub1} could swallow in energy-matters from M_{ub2} over 9 billion years. It can be seen that, the main reason of accelerating expansion of our smaller UBH should have swallowed too much energy-matters from M_{ub2} and got V_{ub12}. The secondary reason might be the space expansion V_{ub13} caused from the expansive speeds V_{ub2} of M_{ub2} after collision. If no that collision happened about 9×10^9yrs ago, the mass of our UBH would be a bit smaller than the original M_{ub1}, but not the current greater M_{ub}; its Event Horizon would be a bit smaller than $2R_{ub1}$, but not the current $2R_{bu}$, because the energy-matters lost by emitting Hawking radiations were extremely little. **Owing to $R_{bu} \approx C\times A_u$, it indicates that, the relative expansive speed of the Event Horizon of our UBH has almost been equal to 2C all the time**. Thus, according to the principle of formula (7), the original mass of our UBH was M_{ub1}, so, $M_{ub1}/ M_{ub} \approx (13.7\times10^9 - 9\times10^9)/ 13.7\times10^9 \approx 4.7\times10^9 /13.7\times10^9 \approx 34.3\%$, correspondingly, the original $R_{ub1}$of M_{ub1}, $R_{ub1}/R_{ub} \approx 34.3\%$; the increased mass after collision from about 9×10^9 years ago to the present is ΔM_{ub}, so, $\Delta M_{ub}/M_{ub} \approx 9\times10^9/13.7\times10^9 \approx 65.7\%$, the increased ΔR_{ub} of ΔM_{ub}, $\Delta R_{ub}/R_{ub} \approx 65.7\%$. Thus, $M_{ub} = M_{ub} = \Delta M_{ub}$, and,

$M_{ub} / M_{ub} \approx 34.3\%$, $R_{ub1}/R_{ub} \approx 34.3\%$

$\Delta M_{ub}/M_{ub} \approx 65.7\%$, $\Delta R_{ub}/R_{ub} \approx 65.7\%$ (10)

VI. The different evolutionary processes and the same destiny of two kinds of black holes (BH),

A. The evolutionary processes of BHs collapsed from compact stars of mass $\geq 3M_0$,

After a compact supernova of mass $\geq 3M_0$ collapsed, its remains would become a BH; all mass should expand full of the whole spherical space of R_b. (annotation: most scientists considered that a Singularity existed at the center of any BH,[9] but author confirmed that no Singularity existed in nature and in any BHs at all.[1][2]). Then, According to Hawking's theory about BHs, if any energy-matters existed outside of a BH, they could be gradually and thoroughly swallowed into BH. In that process, BH would expand its volume and R_b, lower its temperature. Once no more energy-matters outside, BH would only emit Hawking Radiations to outside very slowly and simultaneously shrink its volume as well as raise its temperature. At last, such BH would contract into minimum gravitational BHs (i.e. MGBH, its $M_b \approx 10^{-5}$g, $R_b \approx 1.5\times 10^{-33}$cm, $T_b \approx 0.65\times10^{32}$K, see reference [1]). Once a BH contracted to MGBHs, it could contract its volume no more, but disintegrate and vanish at once at the most fierce burst, because the exclusive forces caused from the highest temperature in MGBHs had been much greater than the gravitational forces of all energy-matters. [10][1][2] It would be the common destiny of all BHs.

According to Hawking's theories about BHs, the lifetime τ_b (from the formation of M_b to MGBH) of a BH (its mass is M_b) is:

$$\tau_b \approx 10^{-27} M_b^3 \text{ (s)} \quad [11][1][2] \qquad (11)$$

For example, if a compact star of mass $\approx 3M_0$ collapsed to a BH and no energy-matters outside to be swallowed in, its lifetime $\tau_b \approx 2\times10^{65}$yrs.

$$d\tau_b \approx 3\times10^{-27} M_b^2 \, dM \text{ (s)} \quad [12][2] \qquad (11a)$$

Suppose our current UBH $M_{ub} = 8.5\times10^{55}$g, stop to expand its volume due to no more energy-matters outside and start to emit Hawking Radiations. After 1 year later, i. e. $d\tau_b = 1$ year, the mass loss dM of our UBH in a year is about: $dM \approx 3\times10^7\times10^{27}/[3\times(8.5\times10^{55})^2) \approx 10^{-74}$g/yr.

B. The evolutionary processes of our real gigantic universal black hole (UBH).

The evolutionary process of our universe as a universal black hole (UBH--**M_{ub}**) is different with above BHs collapsed from a compact star. Author had demonstrated in the past article <New Concepts to Big Bang And Black Holes—Both Had No Singularity at All (Part 1 and part II)>[1][2] that, our universe was born and evolved from the mergence and collision between the large amounts ($N_{ub1} = N_{ub2} = 8.5\times10^{60}$, see IV.) of the same original MGBHs, (its $M_b \approx 10^{-5}$g, $R_b \approx 1.5\times10^{-33}$cm, $T_b \approx 0.65\times10^{32}$K), but not born from Singularity confirmed by the most modern scientists. Just those mergence and collision created the Big Bang of our UBH[1] about 13.7×10^9yrs ago.

The future evolution of our universe as a UBH will depend on whether and how much energy-matters still exist outside of our UBH or no. If no energy-matters exist outside of our UBH at present, our UBH can only emit Hawking radiations, and gradually lose its mass M_{ub} now available. After emitting Hawing radiations in a extremely long period of time, our gigantic UBH will finally contract to MGBHs of 10^{-5}g and instantly vanish at the strongest burst, which will happen after τ_b years, $\tau_b > 10^{-27} M_{ub}^3 = 10^{-27} \times (8.5\times10^{55})^3 > 10^{133}$yrs.

However, our universe has still expanded. It shows that there are large amounts of energy-matters outside of our UBH. Therefore, our UBH will gradually swallow in all energy-matters outside. After that, our UBH will stop to expand and only emit Hawking radiations until it finally contract to become MGBHs of 10^{-5}g and instantly vanish at the strongest burst.

Although two kinds of BHs would have its respective different evolutionary processes, but their final destiny should be the same, they could only contract into MGBHs of 10^{-5}g and instantly vanish at the strongest burst.

C. What did the super-massive BHs (its mass $\approx 10^9 M_\theta$) in the center of every cluster of galaxy come from? They might be all born at the same time with our UBH and formed by much less numbers of the same MGBHs than numbers of our UBH. Just those much smaller BHs as the core of the clusters of galaxies could attract so much energy-matters outside its Event Horizon and swallow in outside energy-matters to become gradually the current super-massive BHs in the universal age A_u. Just their existences and evolutions might lead the appearance of Quasars and lead the galaxy formation in the remote past. Their evolutionary process and final destiny in future will be the same with above two kinds of BHs, but their lifetimes will be between lifetimes of both.

BHs collapsed from compact stars would impossible grow up to a super-massive BH of mass $\approx 10^9 M_\theta$ in our universal age A_u, because each of them does not locate in the center of its cluster of galaxies or galaxy, they are all lack of food. In addition, their ages are all too young.

VII. In our universe, all energy-matter particles always have their respective gravitational forces and the exclusive forces produced by their heat energy, which have been the natural antagonist of gravitational force. Dark energy of exclusive force is not needed and can not really exist.

In our universe, any BHs included our UBH are all the stable entities of extremely long lifetime. Since it is so, the stability and balance inside every BH is very important. In the universe, every energy-matter particle has its mass (gravitational force) and heat energy (temperature) together, even neutrinos and lights are no exceptions (any light has its equivalent mass m_s, $m_s = h/C\lambda$, for heat, $m_s = \kappa T/C^2$). Gravity and heat pressure always form a pair of contradictions co-existed in any particle. Thus, the stability of any BH is the result of the antagonisms and balances between gravitation and heat pressure inside BH.

In any original nebulas or nebulous clusters of our UBH, at any point, under the condition of ideal spherical symmetry, the gas heat pressure P and gravitational force of a particle m_s should be considered as a state of heat-dynamic equilibrium. ρ—density, G—gravitational constant. κ-- Boltzmann's constant, R—distance between the center of M and m_s, M—the total mass in sphere of R,

$dP/dR = -GM\rho/R^2$ [8][2] (12)

$P = n\kappa T = \rho\kappa T/ m_s$ [8][2] (13)

In our universe, since formulas (12) and (13) can be universally applied to the gas states of any galaxies and clusters of galaxies, it doesn't matter whether a super-massive BH may exist in their center or not. **They had been already applied with other equations of border conditions by author to successfully solve many difficult problems in general BHs and our UBH[2].** Both formulas show that, in our UBH, only the exclusive forces of heat energy are always and forever resisting the shrinkage of gravitational forces of energy-matters. Even white dwarfs and neutron stars are all the results of stability between the gravitational forces and heat pressure under some special conditions (see Tolman-Oppenheimer-Volkoff's equation). In the universe, the disintegrated explosions of anything are all the results of its heat pressure inside much greater than its gravitational forces.

In our universe, except gravitational force, there are other three forces, i.e. electric force, weak force and strong force. They may integrate particles into some very solid body in the extremely short distance; such as diamonds, white dwarfs, and neutron stars. However, **only the various balances between**

gravitational force and heat pressure would play a decisive role in the process of universal evolutions. Enough large amounts of energy-matters in high density could crash any solid bodies included neutron stars into particles or continuously collapse into black holes (BH). Once after a big compact star collapsed to a BH, its outside always had much energy-matters for being swallowed in, so, it would no more shrink but conversely expand its volume. **Only the extra-high temperature turned from the gravitational collapse can resist or defeat the further gravitational contraction in BHs.**

In reality, BHs in different size are just the results of the balances between the different heat pressure and the different gravitational force of energy-matter particles.

Thus, in reality, in our universe, the dark energy of exclusive force would not be needed at all for resisting the gravitational force.

VIII. The further analyses and conclusions are demonstrated as below:

Although above demonstrations and calculations are almost qualitative analyses, but not precise and complete quantitative analyses. However, some reliable and significant conclusions can be still drawn out as follows.

A. According to above calculations, under the condition of the current age of our UBH, $A_u = 13.7\times10^9$ years, the **average expansive speed V_{ub1} on the opposite side of Event Horizon of our UBH had almost reached 2C**, i. e. $V_{ub1}\approx 2C$ (on average) or $R_{ub} \le (C\times A_u = R_{uv})$. It shows that, the mass center of our UBH has time enough to transmit its central gravitational forces to the energy-matters on the whole Event Horizon. Thus, ($R_{uv.} = C\times A_u \ge R_{ub}$) is the necessary condition to maintain the stability of our UBH at present, so, our whole UBH inside can keep Flatness and Evenness. Once if $R_{ub} > R_{uv}$, it shows that, the energy-matters of some parts ($R_{ub} - R_{uv}$) in our UBH could not be effected by the gravitational forces of mass center, the whole UBH would not be stable.

B. Two different expansions of our UBH happened in its whole evolutionary process. The expansion of ($V_{ub1} > 2C$) might only happen due to the space expansions of our UBH. For example, $V_{ub1} >> C$ happened at the Inflation just after Big Bang, because at that moment the expansion of our UBH was created by the mergence of large amounts of the same minimum gravitational BHs (i.e. MGBHs, its mass = 10^{-5}g),[1] their instant mergence created the **whole space Inflation** of our new born UBH. It is completely different with the **expansion of Event Horizon of our UBH,** which is caused only by swallowing in energy-matters outside, such expansion **hardly cause ($V_{ub1} > 2C$) as well as ($R_{uv} < R_{ub}$)** [see formula (6b), $V_b \le 2C$].

C. If no collision between our original UBH (M_{ub1}) and M_{ub2} happened about 9 billion years ago and if no energy-matters outside to be swallowed in, M_{ub1} was just an isolated UBH. (a). It could only have a decelerating expansion by the repercussions of initial Inflation after Inflation up to sufficient expansion. Then, R_{ub1} could reach to $R_{ub1} = 2GM_{ub1}/C^2$, $V_{ub1} = 0$**,** and expand no more; but started to shrink its volume by sustained emitting Hawking Radiations. Such conditions should just happen 9 billion years ago, because according to formula (10), $M_{ub1}/M_{ub} \approx 34.3\%$, $R_{ub1}/R_{ub} \approx 34.3\%$. (b). After that, M_{ub1} would extremely slowly decrease its mass until M_{ub1} contracts to MGBHs of 10^{-5}g and instantly vanish at the strongest burst. Thus, **our original UBH (M_{ub1}) would have no way to get the accelerating expansion after Inflation to its vanishing point.**

D. Since $R_{ub} \approx C\times A_u$ is a total value and $V_{ub1} = 2C$ is a average value in our real universal age A_u, as a result, the conditions of ($V_{ub1} > 2C$ or $V_{ub1} < 2C$) could appear in some different special periods**. The space expansion of our UBH exceeding light speed of ($V_{ub1} > 2C$) should happen two times in our universal age A_u. (a).** The greatest accelerating expansion of $V_{ub1} >> 2C$ happened at the instant of Inflation after Big Bang. The space Inflation was created by the mergence of large amounts of the same MGBHs (its mass = 10^{-5}g). (b). $V_{ub1} > 2C$ might happen again in the early period of collision. Just our original smaller UBH--M_{ub1} collided with and dropped in another greater UBH--M_{ub2} about 9 billion years ago, it let M_{ub2} have a great expansive speed V_{ub2}, and lead our M_{ub1} get a ultra space expansive speed V_{ub13}**.** In addition, M_{ub1} could get another expansive speed V_{ub12} due to swallowing in energy-matters from M_{ub2}. Just ($V_{ub13}+V_{ub12}$) could cause the accelerating expansion of our UBH(M_{ub1}) and might let $V_{ub1} > 2C$ (see

paragraph V)**.** Thus it can be seen, **only the mergence or collision of BHs might probably cause the space expansion and probably let the expansive speed ($V_{ub1} > 2C$) sometimes, and might probably create ($R_{ub} > R_{uv}$) temporarily.**

E. (a).Since $V_{ub1}>2C$ had happen two times in our universal age A_u, for keeping $R_{ub} \approx C\times A_u$, [$V_{ub1}<< 2C$ or $V_{ub1} \rightarrow 0$] should have definitely happened in a long-term period before collision of our original UBH(M_{ub1}). **After Inflation, owing to no energy-matters swallowed in by M_{ub1}, M_{ub1} could only do** a **long-term deceleration by the repercussions of initial Inflation all the way** and finally reached the sufficient expansion of R_{ub1} before collision. If $M_{ub1} = 34.3\%M_{ub}$ now available [see formula (10)], R_{ub1} would expand from $R_{ub1} <<<34.3\% R_{ub}$ after Inflation to $R_{ub1} = 34.3\% R_{ub}$ before collision. At the time of $R_{ub1}= 34.3\% R_{ub}$, R_{ub1} had reached to the expansive limit, i.e. $V_{ub1}\approx 0$. Such conditions are with the same of above (a) of C. (b).**Thus, after collision about 9 billion years ago, owing to the space expansion and swallowing in much energy-matters enough caused by collision, V_{ub1} of our UBH (M_{ub}) had to have an accelerating process from ($V_{ub1}\approx 0$) to ($V_{ub1} > 2C$)** for keeping $R_{ub} \approx C\times A_u$**.** (c). Of course, if the accelerating expansion of our UBH (M_{ub}), which was turned from M_{ub1} after collision, reached to the limit of $V_{ub1}>2C$, M_{ub} would be closely followed by a decelerating expansion from the limit of $V_{ub1} > 2C$ to $V_{ub1} \approx 2C$ some time or other, for example, it happened 7 billion years ago. Probably some alternate expansions might happen several times. (d). From then to the present, owing to much energy-matters enough outside to be swallowed in, our UBH could keep the expansive speed of $V_{ub1} \approx 2C$ all the way.

From above D and E, it can be seen, just a long-term deceleration before collision and acceleration after collision had happened, then, the observations could show the obviously accelerating expansion of our UBH about 9 billion years ago and later.

F. If our original UBH (M_{ub1}) at the birth was just equal to the current M_{ub}, i.e. $M_{ub1}= 100\% M_{ub}$ now available, if no collision between UBHs had happened any time and if no energy-matters to be swallowed in all the time; what a evolutionary image of our UBH might happen? (a). The Inflation of our newborn UBH after Big Bang would lead to the Event Horizon R_{uv} of our visible universe much smaller than the original R_{ub} of our UBH, i.e. $R_{uv} <<< R_{ub}$. (b). After that to the present, the deceleration of V_{ub1} from $V_{ub1}>> 2C$ to $V_{ub1} << 2C$ until $V_{ub1}= 0$; so, the original R_{ub} might be the decelerating expansion all the way due to repercussions of initial Inflation until $R_{ub} = 2GM_{ub}/C^2$ and $V_{ub1}= 0$ at present. (c). Owing to the increase of R_{uv} proportional to the growth of our universal age A_u ($R_{uv} = C\times A_u$), it could finally let R_{uv} catch up to R_{ub} of decelerating expansion, i. e. $R_{uv} = R_{ub} = C\times A_u$ and $V_{ub1}= 0$, which was the completely sufficient expansion of R_{ub}. (d). After that, R_{ub} would expand no more, but start to shrink its volume due to emitting Hawking Radiations until the final disappearance. It is said, **in this fictitious model, only the sustained decelerating expansion of our UBH would exist after Inflation to the present due to the repercussions of initial Inflation, no accelerating expansion appeared at any time. It may indirectly indicate that the collision of our UBH (M_{ub1}) with another UBH (M_{ub2}) might truly happen about 9 billion years ago.**

G. Suppose there was other evolutionary mode of my UBH. If our original UBH was extremely small at the birth of the universe, its continuous growth from the birth to the present only depended on swallowing in the very large amounts of energy-matters outside to keep its expansive speed of Event Horizon $V_{ub1} = 2C$ at every moment, i.e. $V_{ub1} \equiv 2C$. Thus, such expansive mode could also maintain $R_{ub} \equiv R_{uv} \equiv C\times A_u = 1.3\times10^{28}$cm at present. However, in this evolutionary mode, no accelerating or decelerating expansions happened, and no Inflation after Big Bang happened too. Therefore, this evolutionary mode did not accord with the real conditions of our UBH, because no Inflation after Big Bang was impossible.

After reviewing the different evolutionary modes of our universe from sections C to G**, only the conditions of section E better accorded with the real evolutionary process of our UBH. It clearly shows that the accelerating expansion of our universe was caused from the collision between two UBHs about 9 billion years ago**.

H. $R_{uv}= C\times A_u = 1.3\times10^{28}$cm, it originates from the supposition of $A_u =13.7\times10^9$ years. However, $R_{ub} = 1.265\times10^{28}$cm, it originates from the supposition of Hubble's constant $H_o = (0.73\pm0.05) \times100 kms^{-1}Mpc^{-1}$. The values of both R_{uv} and R_{ub} are so much close and almost equal to the same value, it let us have no way to judge accurately whether the current expansive speed V_{ub1} of the Event Horizon of our UBH will still keep 2C or has started to slow down to a little slower than 2C. The former case shows that, there will still

be very much energy-matters of M_{ub2} outside for being swallowed in by our UBH at present; the latter case shows that, the energy-matters of M_{ub2} outside of our UBH have reduced [see formulas (6b) and (6c)].

I. The very large amounts of ΔM_{ub} swallowed in from M_{ub2} by our original UBH (M_{ub1}) after collision about 9 billion years ago would completely turn into energy inside our current UBH. However, from formula (10), $\Delta M_{ub} / M_{ub} = 65.7\%$, and $M_{ub1}/ M_{ub} = 34.3\%$. **Have all ΔM_{ub} changed into so-called dark energy of gravitational force in our current UBH? Do all current visible and dark matters of our UBH come from the original UBH M_{ub1}?** How shall we imagine about dark energy**,** if our original UBH--M_{ub1} = 26% $M_{ub} = T^1\mu\nu + T^2\mu\nu$ (see paragraph II), and $\Delta M_{ub} = 74\% M_{ub} = T^3\mu\nu$?

J. No matter how much energy-matters of M_{ub2} remain, but they are limited after all. Once M_{ub2} is completely swallowed in by M_{ub1} in future, then, the future $M_{ub} = M_{ub1} + M_{ub2}$**,** and the future $R_{ub} = R_{ub1} + R_{ub2} = (M_{ub1} + M_{ub2})2G/C^2$. At that time, R_{ub} will reach to complete expansion, $V_{ub} = 0$. Then, M_{ub} will start to contract itself by emitting Hawking Radiations until it finally become MGBHs of 10^{-5}g and at once vanish at the strongest burst.

-----The End------

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Modification of Carboxymethyl Starch as Nano Carriers for Oral Drug Delivery

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Abstract: Received: 8/Natural polymers are considered high value polymeric materials because of their potential as biocompatible materials with medical applications. The chemical modification of natural polymers by grafting has received considerable attention in recent years because of the wide variety of monomers available. As the first part of a continued research on conversion of carboxymethyl starch (CMS) to useful biopolymer-based materials, large numbers of carboxylic functional groups were introduced onto CMS by grafting with poly methacrylic acid (PMAA). Free radical graft copolymerizations were carried out at 70°C, bis-acrylamide as a cross-linking agent and persulfate as an initiator. Equilibrium swelling studies were carried out in enzyme-free simulated gastric and intestinal fluids (SGF and SIF, respectively). This hydrogel converted to nano by freeze drying method and characterized by scanning electron microscopy, differential scanning calorimetry and FT-IR. Two anti-inflammatory model drugs, 5-aminosalicylic acid (5-ASA) and salicylic acid (SA) was entrapped in these nano gels and the in vitro release profiles were established separately in both enzyme-free SGF and SIF. The drug release was found to be faster in SIF. The drug-release profiles indicate that amount drugs release depends on their degree of swelling, and crosslinking. [Nature and Science. 2007;5(3):30-36]. (ISSN: 1545-0740).

Key words: Nano, Modification, CMS, pH-sensitive, Oral drug delivery

Introduction

Nano carriers have important potential applications for the administration of therapeutic molecules. The research in this area is being carried out all over the world at a great pace. Research areas cover novel properties that have been developed increased efficiency of drug delivery, improved release profiles and drug targeting. Although oral delivery has become a widely accepted route of administration of therapeutic drugs, the gastrointestinal tract presents several formidable barriers to drug delivery. To achieve successful colonic delivery, a drug needs to be protected from absorption of the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for colon-targeted delivery of drugs. One strategy for targeting orally administered drugs to the colon includes coating drugs with pH-sensitive hydrogels [1-6]. Polymer bonded drug usually contain one solid drug bonded together in a matrix of a solid polymeric binder. They can be produced by polymerizing a monomer such as methacrylic acid (MAA), mixed with a particulate drug, by means of a chemical polymerization catalyst, such as AIBN or by means of high-energy radiation, such as x-ray or gamma rays [7-9].

Natural polymers have potential pharmaceutical applications because of their low toxicity, biocompatibility, and excellent biodegradability. Starch is the most abundant, renewable biopolymer, which is very promising raw material, available at low cost for preparing of various functional polymers. Carboxymethyl starch (CMS) widely used in pharmaceuticals; however, it may need to be further modified for some special applications. Among diverse approaches that are possible for modifying polysaccharides, grafting of synthetic polymer is a convenient method for adding new properties to a polysaccharide with minimum loss of its initial properties [10]. Graft copolymerization of vinyl monomers onto polysaccharides using free radical initiation, has attracted the interest of many scientists. Up to now, considerable works have been devoted to the grafting of vinyl monomers onto the substrates, especially Starch and cellulose [11]. Existence of polar functionally groups as carboxylic acid need not only for bioadhesive properties but also for pH-sensitive properties of polymer [12, 13]. Because the increase of MAA content in the hydrogels provides more hydrogen bonds at low pH and more electrostatic repulsion at high pH.

It is as a part of our research program on CMS modification to prepare materials with pH-sensitive properties for uses as colon-specific drug delivery. The free radical graft copolymerization poly methacrylic acid onto CMS was carried out at 70 °C**,** bis-acrylamide as a cross-linking agent and persulfate as an initiator. The mixture modified hydrogel and 5-aminosalicylic acid (5-ASA) and salicylic acid (SA) as model drugs were converted to nano by freeze-drying method. The equilibrium swelling studies and in

vitro release profiles were carried out in enzyme-free simulated gastric and intestinal fluids (SGF and SIF, respectively). The influences of different factors, such as content of MAA in the feed monomer and swelling were studied.

Experimental Materials

carboxymethyl starch (CMS) [degree of substitution (DS) = 0.49] was prepared by the method described in the literature [14]. Methacrylic acid (MAA) and bis-acrylamide were purchased from Merck Co. The solvents and reagents were obtained from Fluka. The IR spectra were recorded on a Shimadzu FT IR-408 spectrophotometer. The DSC curves were obtained on a TGA/SDTA 851 calorimeter at heating and cooling rates of 10º C/min under N_2. The amount of released drug was determined on a Philips PU 8620 UV spectrophotometer at the absorption maximum of the free drugs 5-ASA and SA in aqueous alkali (λmax = 205 nm) and (λmax = 235 nm) respectively, using a 1 cm quartz cell. Enzyme-free SGF (pH 1) or SIF (pH 7.4) were prepared according to the method described in the US Pharmacopeia [15].

Methods

Copolymerization: General Procedure

CMS with different molar ratios of methacrylic acid were polymerized at 60-70°C in a thermostatic water bath, bis-acrylamide as a cross-linking agent (CA), using persulfate as an initiator ([I] = 0.02 M) and water as the solvent (50 mL). All experiments were carried out in Pyrex glass ampoules. After the specific time (48 h), the precipitated network polymer was collected and dried in vacuum.

Preparation of nanoparticle

0.5 g of polymer bonded drugs (PBDs) containing 5-ASA or SA was dispersed with stirring in 25 ml deionised water. After approximately 180 min, the PBDs were sprayed into a liquid nitrogen bath cooled down to 77° K, resulting in frozen droplets. These frozen droplets were then put into the chamber of the freeze-dryer. In the freeze-drying process, the products are dried by a sublimation of the water component in an iced solution. Figure 1 show scanning electron microscope (SEM) of nano polymer bonded drugs.

Measurement of swelling ratio

The resulting network polymers swell and become soft in solvents such as H_2O and most organic solvents without dissolving. To measure the swelling, preweighed dry drug-free hydrogels were immersed in various buffer solutions (pH 7.4 and pH 1) at 37º C. After excess water on the surface was removed with the filter paper, the weight of the swollen samples was measured at various time intervals. The procedure was repeated until there was no further weight increase. The degree of swelling was calculated according the relation:

$$SW\ (\%) = [(W_s - W_d)/ W_d] \times 100$$

Where, W_s and W_d represent the weight of swollen and dry samples, respectively. Time-dependent swelling behavior of cross-linked polymers in pH 1 and pH 7.4 at 37º C are plotted in figure 2.

Results and Discussion

To achieve successful colonic delivery, a drug needs to be protected from absorption of the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for colon-targeted delivery of drugs. These requirements have prompted the development of polymeric systems that swell minimally under acidic conditions but extensively in basic intestinal medium.

The composition of the polymer defines its nature as a neutral or ionic network and furthermore, its hydrophilic/hydrophobic characteristics. Ionic hydrogels, which could be cationic, containing basic functional groups or anionic, containing acidic functional groups, have been reported to be very sensitive to changes in the environmental pH. The swelling properties of the ionic hydrogels are unique due to the ionization of their pendent functional groups. The equilibrium swelling behaviour of ionic hydrogels containing acidic and/or basic functional groups is illustrated in Figure 3. Hydrogels containing basic functional groups is found increased swelling activity in acidic conditions and reduced in basic conditions

but on the other hand pH sensitive anionic hydrogels shows low swelling activity in acidic medium but very high activity in basic medium. As shown in Figure 2, an increase in the content of MAA in the feed monomer mixtures resulted in less swelling in SGF but greater swelling in SIF. This is because the increase of MAA content in the hydrogels provides more hydrogen bonds at low pH and more electrostatic repulsion at high pH.

Characterization of hydrolysis product

Polymer–drug adduct (90 mg) was dispersed in 20 ml of pH 8 buffered solution. The reaction mixture was maintained at 37 ºC. After 24 h the hydrolysis solution was sampled and neutralized with 1 M HCl and the solvent was evaporated in vacuo. The resulting crude product was treated with 30 ml of ethyl ether and heated. The suspension was then filtered and the solvent was evaporated under reduced pressure. The residual solid was recrystallized from ethanol and characterized by UV and melting point measurements.

In vitro release studies

Nano and micro polymer bonded drugs (50 mg) were poured into 3 mL of aqueous buffer solution (SGF: pH 1 or SIF: pH 7.4). The mixture was introduced into a cellophane membrane dialysis bag. The bag was closed and transferred to a flask containing 20 mL of the same solution maintained at 37° C. The external solution was continuously stirred, and 3 mL samples were removed at selected intervals. The volume removed was replaced with SGF or SIF. Triplicate samples were used. The sample of hydrolyzate was analyzed by UV spectrophotometer, and the quantity of 5-ASA and SA were determined using a standard calibration curve obtained under the same conditions.

Compare of swelling ratio nano and micro:

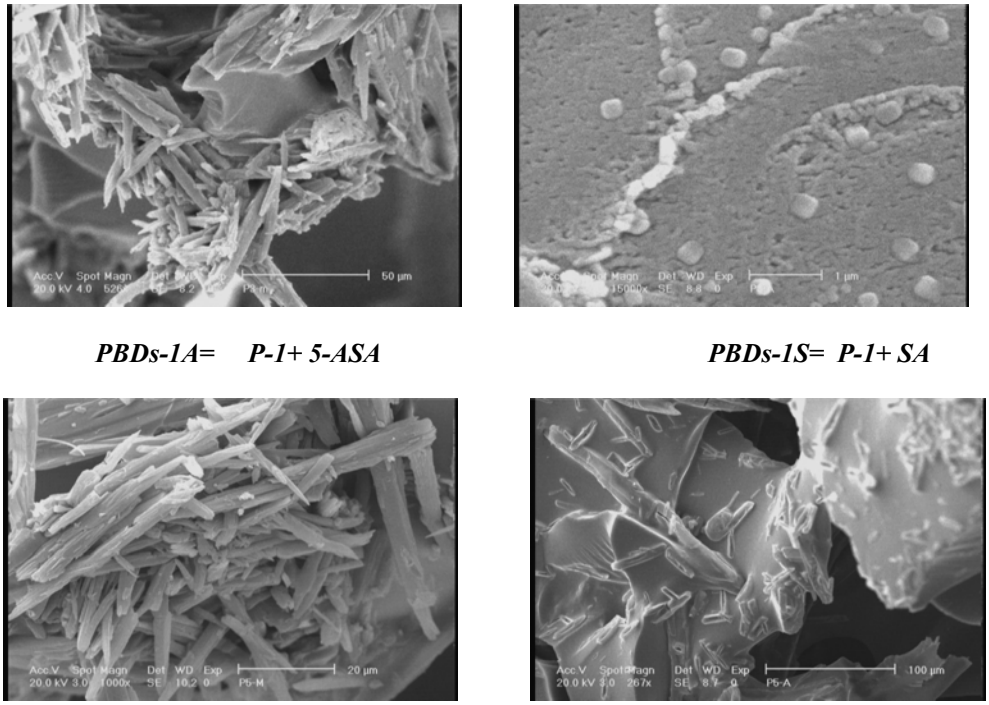
It appears that the degree of swelling depends on their particle size. As shows in fig. 2, a decrease in the molecular size of carriers increased the swelling rate.

Thermal Behavior

The thermal behavior of a polymer is important in relation to its properties for controlling the release rate in order to have a suitable drug dosage form. The glass transition temperature (Tg) was determined from the DSC thermograms. The values are given in Table1. The higher Tg values probably related to the introduction of crosslinks, which would decrease the flexibility of the chains and the ability of the chains to undergo segmental motion, which would increase the Tg values [16]. On the other hand the introduction of a strongly polar carboxylic acid group can increase the Tg value because of the formation of internal hydrogen bonds between the polymer chains.

Drug Release by Hydrolysis of Polymer Bonded Drugs:

For learn of effect of the nature and size of the drug in drug delivery, we study drug release of the polymers containing nano and micro containing 5-ASA and SA as a pharmaceutically active compound as a function of time is shown in figures 4. The concentration of 5-ASA and SA released at selected time intervals was determined by UV spectrophotometry at 205 and 235 nm, respectively. In order to study potential application of PBDs containing 5-aminosalicylic acid and SA as pharmaceutically active compounds, we have studied the drug release behavior of the polymers under physiological conditions. The concentration of drugs released at selected time intervals was determined by UV spectrophotometry. Important parameter for increasing of diffusion coefficient is decreased of particle size. It appears that the degree of drug release polymers depends on their particle size. As shows in 4, a decrease in the molecular size increased the drug release rate. In odder hand, the chemical structure of the drug too is an important factor in hydrolytic behavior of polymeric prodrugs. As shown in Figure 4, High different hydrolysis rate for SA compared to 5-ASA at pHs 1 and 7.4 can be related to the functional groups along the drug. 5-ASA contains both amine (basic) and carboxylic acid (acidic) functional groups. This factor ultimately result in an increase hydrophilicity of 5-ASA in pHs 1 and 7.4, and reduce of different hydrolysis rate 5-ASA compared to SA at pHs 1 and 7.4.



PBDs-1A= P-1+ 5-ASA ***PBDs-1S= P-1+ SA***

PBDs-2A= P-2+ 5-ASA ***PBDs-2S= P-2+ SA***

Figure 1: SEM of nano polymer bonded drugs

Table 1. DSC data and composition of copolymers

Polymers	Molar composition of monomers in the feed				Degree of Substitution (DS) [1]	Tg (° C)
	CMS (gr)	MAA (gr)	CA (gr)	IN (gr)		
P-1	1	3	0.05	0.05	0.49	130
P-2	1	2	0.05	0.05	0.49	142

*1: **the method described in the literature [14].***

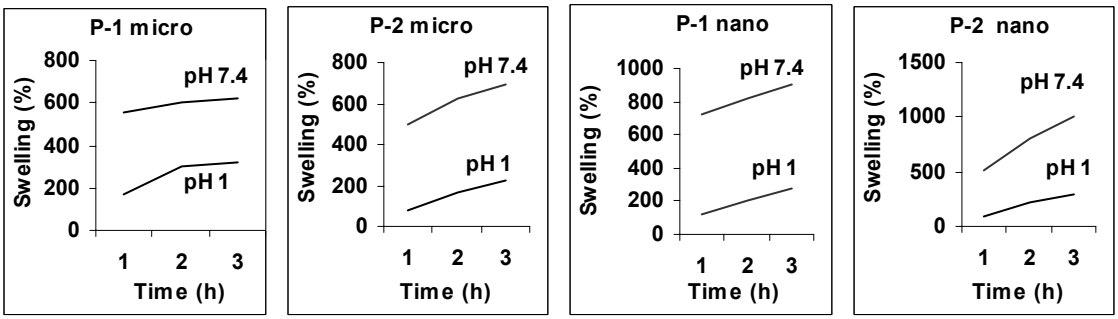


Figure 2. Time-dependent swelling behavior of micro and nano carriers as a function of time at 37ºC.

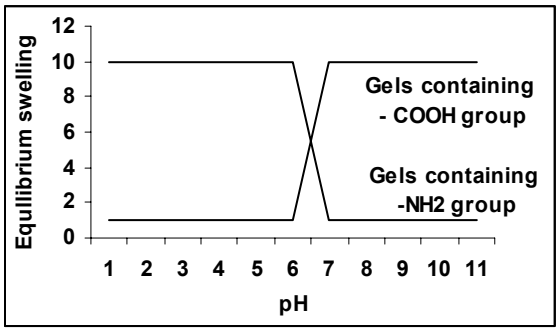
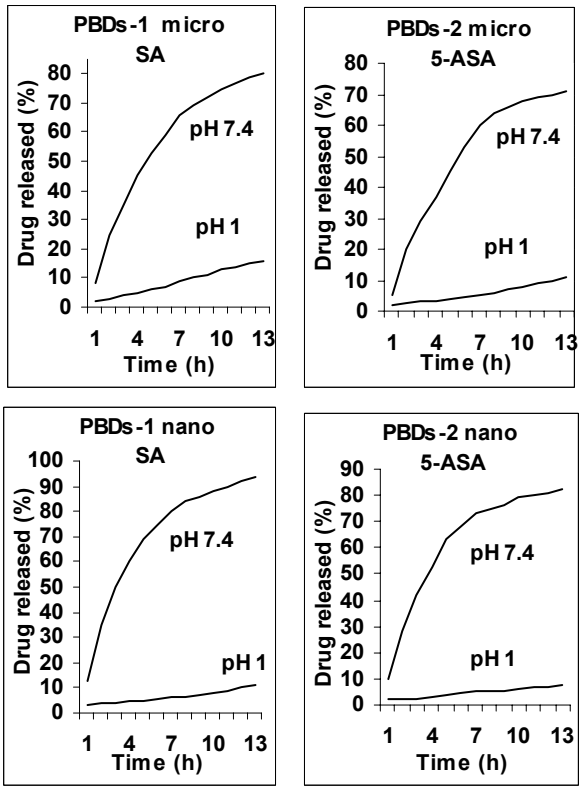


Figure 3. Equilibrium degree of swelling in response to pH



*F*igure 4. Release of drug from micro and nano polymeric carriers as a function of time at 37ºC.

Conclusion

The size and the nature of the incorporated drug play a very important role in determining the efficiency of its release from the carrier. An increase in the molecular size of the drug or PBDs reduces the drug release rate [17]. The swelling and hydrolytic behavior of the hydrogels was dependent on the content of MAA groups and caused a decrease in gel swelling in SGF or an increase in gel swelling in SIF. Modified CMS with different contents of MAA and CA by graft copolymerization reactions were carried out under microwave-radiation. The swelling of the hydrogels was dependent on the content of MAA groups and caused a decrease in gel swelling in SGF or an increase in gel swelling in SIF. Incorporation of MAA made the hydrogels pH-dependent and the transition between the swollen and the collapsed states occurred at high and low pH. The swelling ratios of the hydrogels increased at pH 7.4, but decreased at pH 1 with increasing incorporation of MAA. In odder hand, the drug release rate of the PBDs related to

particle size of carrier and chemical structure of the drug in polymer. That increased by reducing of particle size. Based on the great difference in hydrolysis rate at pH 1 and 7.4, this modified natural polymer appears to be good candidates for colon-specific drug delivery.

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Received: 8/9/2007

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Comparative X-Ray Structure analysis of systemic fungicides (N-(2,6 dimethyl phenyl)-N-(2-keto-1-methyl butyl) 3-hydroxypropanamide) AND cis N-(1,1,2,2-tetrachloroethylthio)-4-cyclohexene-1,2-dicarboximide)

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Abstract: There are large numbers of chemicals compounds for the protection of crops, available commercially in the market but their effects dependent on the climate, type of soil, and other physical parameters. The interactions of proposed fungicides with the macromolecule of the parasite are dependent on the stereochemistry of these compounds. In order to design more effective synthetic fungicides, it is necessary to analyze the three dimensional structure of these compounds and if possible the receptor molecule. The structures of these compounds can be obtained by X-ray diffraction method in crystalline form and they will invariably be similar to their structures in solution. The composition of the crystal **(N-(2,6 dim ethyl phenyl)-N-(2-keto-1-methyl butyl) 3- hydroxypropanamide)** is confirmed by comparing the infra-red spectra of the two components. The unit cell parameters are a =7.865(1)Å, b =13.122(2)Å, c =15.130(1)Å, α =90(1)^o, β=101.75(2)^o, γ=90(1)^o. The space group is determined to be $P2_1/c$. The calculated density of the crystal is 1.1919g/cm^3 and measured density is 1.192g/cm^3.All the lengths in the Benzene ring vary from 1.3705(2)Å to 1.4176(1)Å, show a good agreement with their standard value of 1.395Å. The Unit cell parameters of **cis N-(1, 1 ,2,2-tetrachloroethylthio)-4-cyclohexene-1 ,2-dicarboximide)** are a=10.5665(7)Å, b=6.6413(3)Å, c=19.3973(12)Å and Z=4. Thus the space group is determined to be $P2_1/c$ and crystal of monoclinic system. We can see that there are some differences in unit cell parameters in both the crystals. We will see how these differences affect the systemic fungicides biological activity. We compare the structures of both the systemic fungicides. [Nature and Science. 2007;5(3):37-43]. (ISSN: 1545-0740).

Keywords: X-ray crystallography, Triagonal Hybridization, systemic fungicides

I. Introduction

FUNGICIDES: Important class of chemicals/ drugs used widely for the protection of crops.

Structure-Activity Relationship

The activity of a drug is intimately related to its chemical structure. Knowledge about the chemical structure of a chemical is useful for:-

1. Synthesis of new compounds with more specific actions and fewer adverse reactions.
   (a) To increase/decrease the duration of action of the original drug or to get a more potent compound.
   (b) To restrict the action to a specific system of the body.
   (c) To reduce the adverse reactions, toxicity and other disadvantages associated.
2. Synthesis of competitive antagonists.
3. Understanding the basic chemical groups responsible for drug action.

Action of Systemic Fungicides

Very little is known about the mechanism of these fungicides. The following are the possibilities:-

1. Inactivation of the enzymes and toxins of the pathogens.
2. Selective accumulation of the fungicide due to greater permeability of the fungus cell wall.
3. Damage to the membranes of the fungal hyphens and inhibition of structures, such as aspersoria, cushion formation emergence of germ tubes and formation of haustoria's.

4. Inhibition of fungal enzymes or their destruction, Systemic fungicides are more specific in their action than non-Systemic fungicides.

II. Experiments

Crystals of **(N-(2,6 dimethyl phenyl)-N-(2-keto-1-methyl butyl) 3-hydroxypropanamide)** are grown at 4^o-5^o from its solution in Toluene by slow evaporation method Crystallization of **cis N-(1, 1 ,2,2-tetrachloroethylthio)-4-cyclohexene-1 ,2-dicarboximide)** is done by slow evaporation from a solution of methyl alcohol at 40°C temp. The crystals found were pale yellow in color and rectangular in shape.. The unit cell parameters are determined directly by automatic computerized 4 - circled Enraf Nonious CAD-4 diffractometer in ω-2θ scan mode.

DATA COLLECTION AND STRUCTURE SOLUTION: The three dimensional intensity data are collected on a computerized automatic 4-circled CAD-4 Enraf-Nonious diffractometer and the crystal structure is solved using the SHELXS-97.

Refinement: The structure determination is carried out on VAX machine using SHELXS-97 program. All the non hydrogen atoms are located in the beginning itself. The co-ordinates thus obtained are fed to SHELXL-97 for refinement. The final R index is 0.045 for all the observed reflection 3849 (including all the unique reflections) for **(N-(2,6 dimethyl phenyl)-N-(2-keto-1-methyl butyl) 3-hydroxypropanamide).** For **cis N-(1,1,2,2-tetrachloroethylthio)-4-cyclohexene-1,2-dicarboximide)** the R factor dropped to 0.0516 after several cycles of refinement. To reduce R factor to 0.0437, further refinement of the structure was carried out with individuals' anisotropic temperature factors exponent of the form.

$-2Pi\ ^\ 2[h^2a^{*2}U_{11}+----------+2hKa^*b^*U_{12}]$

The hydrogen atoms are fixed by geometrical consideration at this stage, but not included in refinement. Refinement of the structure is terminated after two more cycles when all the shifts in Parameter's become much smaller than the corresponding estimated standard deviations. The final R value is 0.0437 for all the 8018 reflections for **cis N-(1, 1 ,2,2-tetrachloroethylthio)-4-cyclohexene-1 ,2-dicarboximide).**

III. Result And Discussion

The ORTEP diagram of **(N-(2,6 dimethyl phenyl)-N-(2-keto-1-methyl butyl) 3-hydroxypropanamide)** is shown in **fig 1** and the ORTEP diagram of **cis N-(1, 1 ,2,2-tetrachloroethylthio)-4-cyclohexene-1 ,2-dicarboximide)** is shown in **fig 2**. Bond length for **(N-(2,6 dimethyl phenyl)-N-(2-keto-1-methyl butyl) 3-hydroxypropanamide)** is given in **Table 1** and Bond Angles in **Table 2.** Bond length for **cis N-(1, 1 ,2,2-tetrachloroethylthio)-4-cyclohexene-1 ,2-dicarboximide)** is given in **Table 3** and Bond Angles in **Table 4. In N-(2,6 dimethyl phenyl)-N-(2-keto-1-methyl butyl) 3-hydroxypropanamide)** the geometry around N(1), C(13) and C(10) appears to be normal as all the lengths are close to single bond normal valuesand the angles are according to the configuration. The C-N distances are similar to that observed in structures having triagonal hybridization.The equations for the mean planes were calculated by the method suggested by Blow (1960). All the lengths in the Benzene ring vary from 1.3705(2)Å to 1.4176(1)Å, show a good agreement with their standard value of 1.395Å. The deviations of the inner bond angles in the Benzene ring from 120º are slightly greater than 2σ (=0.7º).The geometry around N(1), C(13) and C(10) appears to be normal as all the lengths are close to single bond normal values and the angles are according to the configuration. The C-N distances are similar to that observed in structures having triagonal hybridization.It is of interest to see in **cis N-(1,1,2,2-tetrachloroethylthio)-4-cyclohexene-1,2-dicarboximide)** the geometry of Phthalimide group. The C (1)-C (2) bond length is much shorter 1.309(4) Å compared to standard values, whereas the largest bond distance is C (5)-C (6) of 1.543(3)Å But as far as bond angles are concerned, they vary from 110.7 (2) ° to 120.2(3) °, thus suggest that the ring is compressed as expected. The five-member ring shows usual behavior. The geometry around S (1), C (10) and C(12) appears to be normal as all the lengths are close to single normal bond values and angles are according to the configuration. The N (8)-S(1)-C(1O) angle of 102.2(9)° shows that the chain is almost right angle to phthalimide group. The angle of twist between phthalimide group and remaining chain N (8)-S (1)-C (10)-C (11) is of -74.9(2) °. The phthalimide

group appears to be planner, as we calculated mean planes using Blow's method. If we look to the angles between different planes, it appears that the molecule is highly twisted and folded.

Table 1. Bond distances in {A} involving non -hydrogen atoms with estimate standard deviations in parentheses

O(1)	- C(10)	1.2191(1)
O(2)	- C(14)	1.1953(2)
O(3)	- C(11)	1.2382(2)
N(1)	- C(1)	1.4429(2)
N(1)	- C(10)	1.3618(1)
N(1)	- C(13)	1.4680(1)
C(1)	- C(2)	1.4056(2)
C(1)	- C(6)	1.4176(1)
C(2)	- C(3)	1.3972(1)
C(2)	- C(8)	1.4987(1)
C(3)	- C(4)	1.3811(2)
C(4)	- C(5)	1.3705(2)
C(5)	- C(6)	1.3900(1)
C(6)	- C(7)	1.4859(2)
C(10)	- C(20)	1.5530(2)
C(11)	- C(20)	1.3919(1)
C(13)	- C(14)	1.5112(2)
C(13)	- C(17)	1.5321(1)
C(14)	- C(15)	1.3435(1)
C(15)	- C(16)	1.4389(2)

Table 2. Bond angles {A} of non-hydrogen atoms with estimated standard deviations in parentheses

C(1)	- N(1)	- C(10)	121.43(1)
C(1)	- N(1)	- C(13)	120.97(1)
C(10)	- N(1)	- C(13)	116.33(2)
N(1)	- C(1)	- C(2)	118.39(1)
C(2)	- C(1)	- C(6)	121.87(1)
N(1)	- C(1)	- C(6)	119.73(2)
C(1)	- C(2)	- C(3)	117.40(2)
C(1)	- C(2)	- C(8)	121.63(2)
C(3)	- C(2)	- C(8)	120.93(2)
C(2)	- C(3)	- C(4)	121.54(2)
C(3)	- C(4)	- C(5)	119.86(1)
C(4)	- C(5)	- C(6)	122.12(2)
C(1)	- C(6)	- C(5)	117.15(1)
C(5)	- C(6)	- C(7)	121.05(1)
C(1)	- C(6)	- C(7)	121.80(1)
O(1)	- C(10)	- N(1)	122.35(1)
O(1)	- C(10)	- C(20)	121.96(2)
C(10)	- N(1)	- C(13)	116.33(1)
O(3)	- C(11)	- C(20)	113.99(2)
N(1)	- C(13)	- C(14)	111.32(1)
C(14)	- C(13)	- C(17)	108.37(1)
N(1)	- C(13)	- C(17)	112.44(2)
N(1)	- C(13)	- C(17)	112.44(1)
O(2)	- C(14)	- C(13)	126.87(1)
C(13)	- C(14)	- C(15)	109.26(1)
C(14)	- C(15)	- C(16)	116.79(2)

C(10) - C(20) - C(11) 112.98(2)

Table 3. bond length [A] with estimated standard deviation in parenthesis for cis N-(1, 1 ,2,2-tetrachloroethylthio)-4-cyclohexene-1 ,2-dicarboximide

S (1) - N (8)	1.6854 (17)
S (1) - C (10)	1.820 (2)
C1 (1) -C (10)	1.773 (2)
C1 (2) - C (10)	1.767 (2)
C1 (3) - C (11)	1.764 (2)
C1 (4) - C (11)	1.768 (2)
C (1) - C (2)	1.309 (4)
C (1) - C (6)	1.491 (4)
C (3) - C (4)	1.534 (3)
C (4) - C (9)	1.502 (3)
C (4) - C (5)	1.533 (3)
C (5) -C (7)	1.517 (3)
C (5) -C (6)	1.543 (3)
C (7) - O (13)	1.196 (3)
C (7) - N (8)	1.414 (3)
N (8) -C (9)	1.400 (3)
C (9) -C (12)	1.203 (3)
C (10) - C (11)	1.537 (3)

Table 4. Bond Angle [Degree] with estimated standard deviation in parenthesis

N (8) -S (1) - C (10)	102.23 (9)
C (2) -C (1) -C (6)	120.2 (2)
C (2) -C (1) -H (1)`	119.9
C (6) - C (1) - H (1)	119.9
C (6) -C (1) - C (3)	120.1 (2)
C (1) - C (2) - H (2)	120.0
C (1) - C (2) - H (2)	120.0
C (2) - C (3) -C (4)	110.7 (2)
C (2) - C (3) - H (3A)	109.5
C (4) - C (3) -H (3A)	109.5
C (2) - C (3) -H (3B)	109.5
C (4) - C (3) -H (3B)	109.5
H (3A) -C (3) - H (3B)	108.1
C (9) - C (4) - C (5)	105.33 (16)
C (9) - C (4) - C (3)	109.33 (18)
C (5) -C (4) - C (3)	114.73 (18)
C (9) - C (4) -H (4)	109.0
C (5) - C (4) -H (4)	109.0
C (3) -C (4) - H (4)	109.0
C (7) -C (5) - C (4)	105.50 (16)
C (7) -C (5) -C (6)	110.37 (19)
C (4) -C (5) -C (6)	114.00 (19)
C (7) -C (5) -H (5)	108.9
C (4) -C (5) -H (5)	108.9
C (6) -C (5) -H (5)	108.9
C (1) -C (6) -C (5)	111.56 (19)
C (1) -C (6) -H (6A)	109.3
C (5) -C (6) -H (6A)	109.3

C (1) -C (6) -H (6B)	109.3
C (5) -C (6) -H (6B)	109.3
H (6A) -C(6) -H (6B)	108.0
O (13) -C (7) -N (8)	124.32 (19)
O (13) -C (7) -C (5)	127.97 (19)
N (8) -C (7) -C (5)	107.71 (17)
C (9) -N (8) -C (7)	112.26 (17)
C (9) -N (8) -S (1)	123.74 (14)
C (7) -N (8) -S (1)	122.99 (14)
O (12) -C (9) -N (8)	123.40 (19)
O (12) -C (9) -C (4)	127.6 (2)
N (8) -C (9) -C (4)	108.95 (17)
C (11) -C (10) - C1(2)	109.07 (15)
C (11) -C (10) - C1(1)	110.81 (15)
C1 (2) -C (10) - C1 (1)	109.99 (12)
C (11) -C (10) -S (1)	113.91 (15)
C1 (2) - C (10) -S (1)	110.64 (11)
C (1) -C (10) -S (1)	102.26 (11)
C (10) -C (11) -C1 (3)	111.09 (16)
C (10) -C (11) -C1 (4)	111.84 (16)
C1 (3) -C (11) -C1 (4)	109.11 (13)

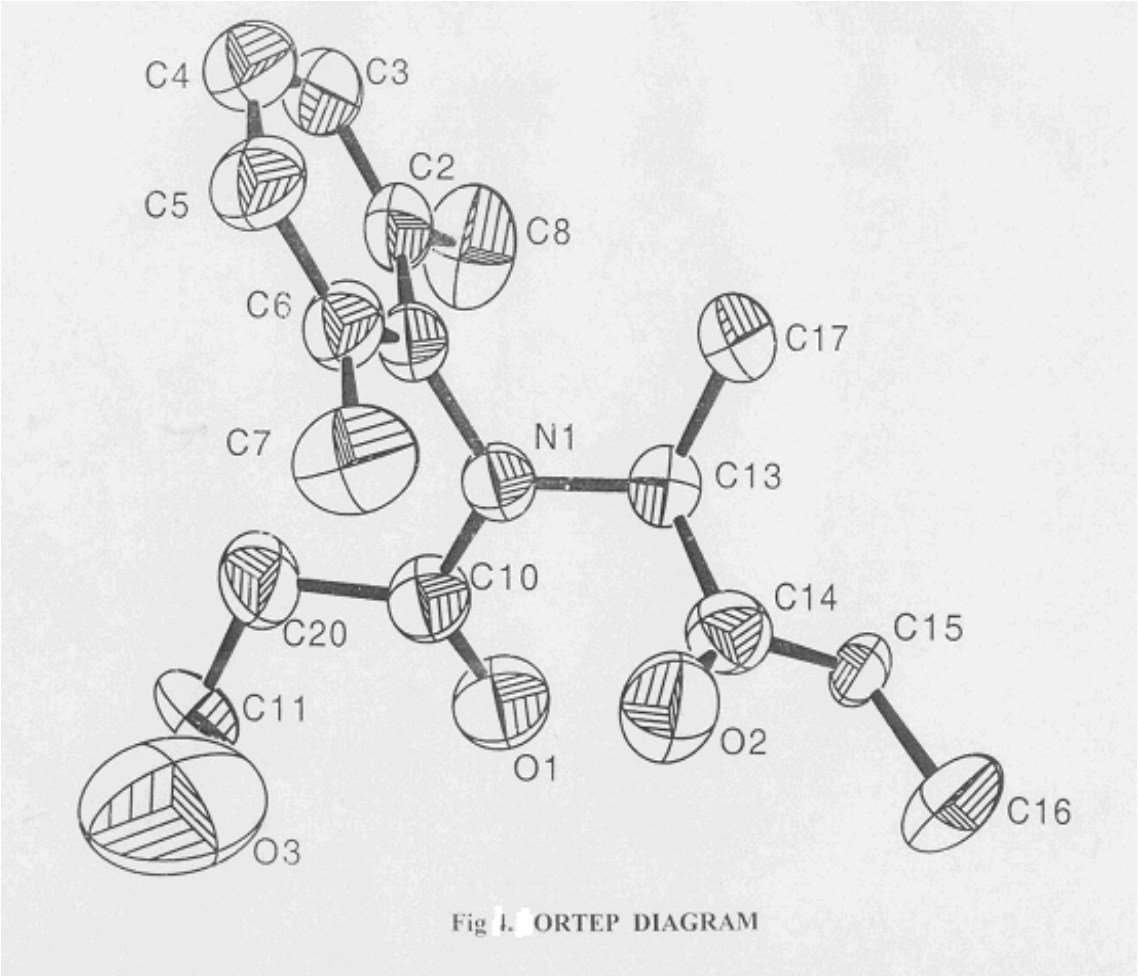

Fig 1. ORTEP DIAGRAM

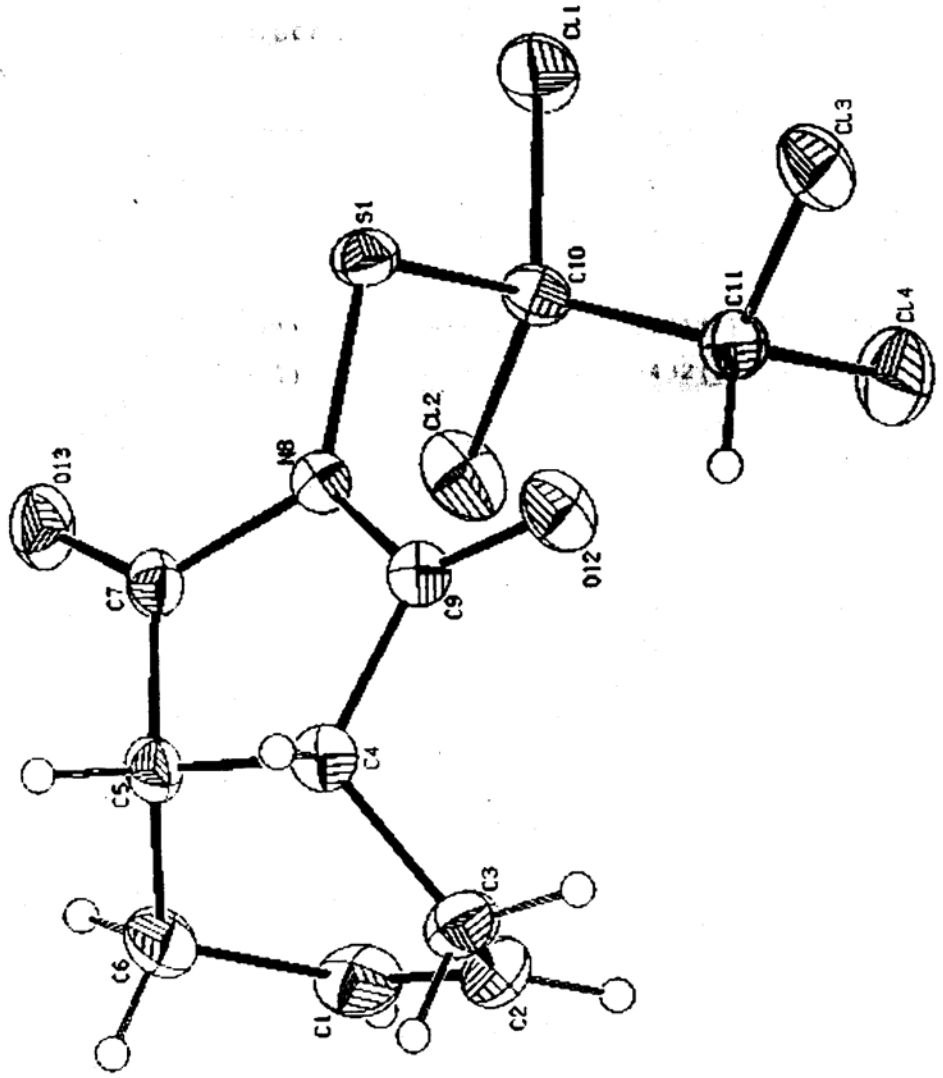

Fig. 2: ORTEP for cis-N-(1,1,2,2-tetrachloroethylthio)-4-cyclohexene-1,2-dicarboximide

Acknowledgement

The Financial assistance provided by Deptt of Science and Technology (D. S. T), New Delhi in form Junior Research Fellow is gratefully acknowledged. Iam thankful to Prof T. P Singh Head Deptt of BioPhysics, AIIMS, New Delhi for providing me National Facility CAD-4Diffractometer and Lab. Iam also thankful to Prof. D. Vellmurgan, UNIV OF MADRAS for his valuable help in Data collection.

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Antimicrobial Susceptibility and Beta-lactamase detection of MRSA in Osogbo. SW Nigeria

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ABSTRACT： A total of 156 *Staphylococci aureus* isolates from different human clinical specimens comprising urine, stool, skin, wound swabs, ear and nose / nasal swab and blood obtained from patients at two large referral hospitals in Osogbo, Nigeria were screened for their antibiograms and plasmid profiles. A total of six antibiotic resistance profiles were obtained with ten of the isolates showing multi-drug resistance. Plasmids of three size ranges were detected in the isolates. Isolates with high multi-drug resistance profiles were found to possess multiple plasmids with large sizes in the range of 6.5 – 23.2kb. Very high resistance levels (87.5%) were detected against penicillin and tetracycline while vancomycin and gentamicin recorded the least resistance levels of (62.5%) and (6.3%) respectively among the isolates. The starch paper analysis confirmed the presence of beta-lactamase production in all the isolates tested. [Nature and Science. 2007;5(3):44-48]. (ISSN: 1545-0740).

Keywords: Antibiogram; plasmid profile; clinical specimen.

Introduction

Humans are a natural reservoir for *S. aureus*, and asymptomatic colonization is far more common than infection. Colonization of the nasopharynx, perineum, or skin, particularly if the cutaneous barrier has been disrupted or damaged, may occur shortly after birth and may recur anytime thereafter, Payne, et al., (1966). Infections caused by *Staphylococcus aureus* resistant to methicillin (MRSA) are increasing in prevalence in adults and children. Nosocomial infections account for morbidity and mortality of millions of patients annually, worldwide. *Staphylococcus aureus* especially Methicillin-resistance *S. aureus* (MRSA) is relatively ubiquitous and is the cause of many community, endemic and epidemic nosocomial colonization and infections (Mansouri et. al., 1997). MRSA is of concern not only because of its resistance to Methicillin but also because it is generally resistant to many other chemotherapeutic agents. Its now a major nosocomial pathogen in hospitals today due to prolonged hospital stay, intravenous drug abuse, and carriers of MRSA, especially in the nasal cavity, even along medical personnel (Vidhani et. al.,2001). Community-acquired MRSA infections in the absence of identified risk factors have been reported infrequently (Mansouri. et al., 1997). This study was undertaken to document the prevalence of methicillin resistance among *S.aureus* isolates at tertiary care teaching hospital, and even among healthy individual in this region. The pattern of susceptibility of both methicillin sensitive and methicillin resistance isolates to the commonly used antimicrobials were also analysed. The betalactamase production of this bacteria was also investigated using starch paper method. This work deals with prevalence of MRSA in a tertiary institution and its beta-lactamase production.

Materials and Method

This study was carried out in Osogbo, Ladoke Akintola University of Technology College of Health Sciences, South Western Nigeria, from August-2004 to October 2004, we processed the samples of Pus, Urine, Blood, high vaginal swabs, Sputum, throat swabs, and ear swabs received from patients and

healthy individuals. A total of 106 Staphylococci were isolated out of which 28 were from healthy individuals. The isolates were confirmed by standard microbiological procedures (Cowan and Steel, 1954)

Laboratory Methods

Specimens were screened by preliminary Gram's stain and were inoculated on 10% sheep blood agar and MacConkey's agar. Staphylococcus aureus was identified by conventional techniques Layton et al., (1995). Antimicrobial sensitivity was performed on all the *Staphylococcus aureus* isolates by Kirby-Baur's disc diffusion method (Duguide et al., 1996). Oxacillin sensitivity was performed on Muller-Hinton agar with 4% sodium chloride. The strains were reported as sensitive, or resistant, to Oxacillin with inhibition zone diameter equal or more than 13mm and less than or equal to 10mm respectively. Disk diffusion testing was performed as recommended by the National Committee for Clinical Laboratory Standards; briefly, a broth culture suspension of the isolate to be tested was prepared in trypticase soy broth and turbidity adjusted to a 0.5 McFarland standard. The zone sizes were read after 24 hours of incubation in ambient air at 35°C. Isolates were classified as either susceptible or not susceptible Bauer et al. (1966). All the resistant strains were subjected to testing for beta lactamase production by starch paper method. NCCCL (1993). The MRSA isolates were tested for susceptibility to the following additional antibiotics: clindamycin, erythromycin, gentamicin, penicillin, tetracycline and vancomycin.

Results

Out of 156 isolates of Staphylococci collected, 28 (17.9%) were from healthy individuals. The results showed 67 were coagulase positive and 89 (57.1%) were coagulase negative. Out of 67(42.9%) coagulase positive Staphylococci 32 (47.8%) isolates were resistant to Methicillin. Table 1 shows the prevalence distribution of various clinical specimens of samples with methicillin resistance staphylococcus aureus and coagulates positive *staphylococcus aureus*. Highes prevalence of samples was isolated from wound from both MRSA and COSA, while stool sample had the least distribution for both. Table 2 shows the antibiotic resistance pattern of Methicillin resistant and sensitive strains of *Staphylococcus aureus.* As many as ten (14.9%) Staphylococcus strains were resistant to all antibiotics tested and three (4.4%) were resistant to all other antibiotics except Methicillin. Multidrug resistance was found to be less common amongst the methicillin sensitive Staphylococcci aureus (MSSA) strains. Maximum resistance was observed against tetracycline in this study and penicillin, (87.5% %) respectively. Least resistance was observed against gentamicin (62.5%) and vancomycin (6.3%) among MRSA. MIC values to Oxacillin > or equal to 250ugm/ml were found only in one strain, while majority of the strains MIC ranged between 2ugm/ml to 10 ugm/ml. Although the mean value of majority of the strains (21 out of 32) showed MIC values of 6ug/ml.

Table 1. Prevalence Rate Of Mrsa And Cosa From Clinical Specimen

SOURCE	Coagulase positive Staphylococcus aureus	MRSA (%)
SKIN	12	6 (9.0)
BLOOD	I4	6 (9.0)
STOOL	3	2 (3.0)
URINE	6	1 (1.5)
WOUND SWAB	18	13(19.4)
EAR SWAB/ NASAL SWAB	14	4 (5.9)
TOTAL	67	32 (47.8%)

Table 1 b. Prevalence rate of MRSA

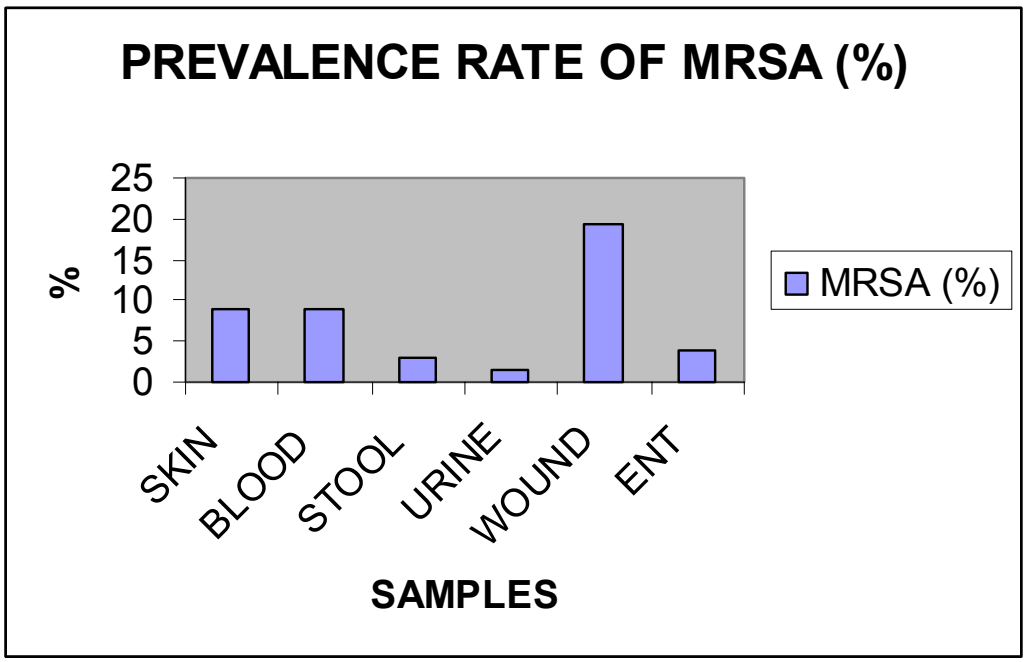

Table 2. Antibiotics

ANTIBIOTICS	MRSA (N=32)	MSSA (N=35	P.VALUE	
PENICILLIN	28 (87.5%)	24 (68.6%)	>0.05	NS
ERYTHROMYCIN	26 (81.1%)	20 (57.1%)	<0.05	NS
GENTAMICIN	20 (62.5%)	16 (45.7%)	<0.05	NS
CHLORAMPHENICOL	24 (75%)	19 (54.35)	>0.05	S
TERACYCLIN	28 (87.5)	12 (34.3%)	0.05	S
VANCOMYCIN	2 (6.3%)	--1 (2.9%)	0.05	Significant

P.Value = Indicates statistically significance difference between methicillin resistance and sensitive strains.
NS= NOT SIGNIFICANT
S= SIGNIFICANT

Discussion

Because of the ability of staphylococci to change over time, the MRSA will continue to be a problem in the future, as it has been in the past and still is, at present. Despite, intensive efforts to control resistant organisms by aggressive infections control methods antibiotic-resistant Staphylococci, especially MRSA has become the most common cause of hospital acquired infections worldwide. Chaudhary et al., (1999); Anupurba et al., (2003); (Taiwo et al., 2005). In this study, the frequency of MRSA was determined, (47.8%), which is almost similar but higher to other cases reported in pervious findings by (Taiwo et al., 2005) yet it is very high in comparison to the records of Zaman et al., (1994), Majumder et al., (2001) and Anupurba., (2003)**.** The injudicious use of antibiotics in Osogbo public hospitals and because of the easy availability of antibiotics without prescription, the chances of the emergence of resistant strains is enhanced. Lack of public awareness has contributed to the degeneration of the situation. Many investigators have reported an increase in the incidence of MRSA during recent years, most of which originated from wounds (pus). Vidhani et a.l (2001). We also found a high rate of MRSA isolates i.e. thirteen (40.6%) from the clinical specimens also showed multiple drug resistance. In our study one (2.9%) MSSA (Methicillin sensitive *Staphylococcus*

aureus) isolates were resistant to all other antibiotics tested. We have observed in our study that resistance to different antibiotics among MRSA strains was significantly higher than those, which were sensitive to methicillin. The values of MIC observed in Oxacillin resistance is almost similat to previous work of (Tahnkiwale et al., 2002). Vancomycin which was proposed as the drug of choice in several chronic cases of MRSA seems to be a better drug when compared to other antibiotics used in this study though with a low resistance of (6.3%) was observed for MRSA. The rapid detection of colouration by the starch paper method by all the isolates tested, confirmed the presence of beta-lactamase in all the isolates tested, and further confirmed the indiscriminate use of antibiotics in this area. This is perhaps due to the different clonally expansion and drug pressure in the community.

Since complete eradication of MRSA may not be possible, control of transmission seems to be the appropriate goal. The efficacy of some controlling methods are widely recognized and recommended by most authors. The first and the most effective way among these are to avoid transmission through hand contamination from personnel even to patients. The use of broad-spectrum antibiotics for treating infections also increases the rate of MRSA and other resistant bacteria. Therefore chemotherapy should be guided by sensitivity of the probable causative organism. Accurate detection of MRSA by clinical laboratories is of great importance, also awareness should be created about the route of its transmission in the community and the risk factors for infection such as antimicrobial and parental drug use.

Conclusion

Abused and injudicious use of antibiotics will lead to development of drug resistance. Also there is resistance to other antibiotics in Methicillin resistant strains. Timely detection of methicillin resistant strain will help in prevention of hospital-acquired infections. Control of MRSA infections is essential, and it can be achieved by proper implementation of hospital control measures and regular surveillance activity for proper documentation and control measures aimed at combating spread and control.

Received: 9/16/2007

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Evaluation Of Measles Vaccines In Northeastern Nigeria

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ABSTRACT: Measles account for nearly half of the 1.7 million annual deaths due to childhood vaccine-preventable diseases. This study was designed to assess the sero-conversion rate of a single dose measles vaccine on children aged 9-12 months. The pre and post measles vaccination sera of the children as well as sera of some of the vaccinees' mothers were tested using the heamagglutination inhibition test. Of the 136 prevaccination sera, 26 (16.9%) had measles HI antibody with Geometric mean titer (GMT) of 36.4 while only 22 (23%) of 100 post vaccination sera had antibody against measles virus and having a GMT of 22.9. The measles HI antibody between the mothers' and pre vaccination sera of the children was not significantly different (Kw> 3.000 df. 2, P= 0.022313). However, five mothers and their corresponding children had measles antibody with the GMT of 21.1 and 9.2 respectively. Also, five seropositive mothers (GMT= 55.7) had seronegative children. Nevertheless, 11 of 60 (18.3%) seronegative mothers had seropositive children with GMT 14.1. Ten children (14.3%) had clinical measles infections before they were brought for vaccination at the age of 9 months. In addition 13 (18.6%) had index cases of measles in their families. No significant difference was observed between measles antibody (pre-vaccination) and previous clinical measles infection (t= 0.23730, df. 24, P= 0814519). Although varied titers of measles vaccines, (ranging from $10^{1.5}$ to $10^{6.0)}$ were observed in all the centers chosen for the study. There was however no significant difference in titres among the various vaccination centres ($P > 0.05$). There is need to give quality measles vaccine whether single or supplementary dose if the global effort to reduce measles by 95% in Africa is to be achieved. [Nature and Science. 2007;5(3):49-53]. (ISSN: 1545-0740).

Keyword: Measles, vaccines, children, Nigeria

1. INTRODUCTION

It was observed that countries with a single-dose on measlesare not only the poorest and least developed, but report the lowest routine vaccination coverage and experience the highest measles diseases burden. (International Note 2002). Yet not all children who receive a single measles vaccine at 9 months of age will develop a protective response and are the primary vaccine failure (WHO 2001). These authors attributed primary vaccine failure to the presence of maternal antibody at the time of vaccination, damaged vaccines; receipt of immune globulin, genetic factors and other incompletely understood factors (Meissner et al 2004). Therefore a small proportion of individual who remain susceptible due to primary vaccine failure will accumulate over time. When exposure occurs, the contagiousness of measles virus may result in an outbreak even when only a small number of case contacts are susceptible. It was then concluded that prevention of endemic measles transmission is not possible in countries with a single-dose immunization program, even when vaccination rates approach 100% (Meissner et al 2004). This study was designed to assess the sero-conversion rate of a single dose measles vaccine on children aged 9-12 months.

2. MATERIALS AND METHODS

STUDY POPULATION:

Children aged 9 months and above attending immunization centers such as Specialist Hospital (SH), University of Maiduguri Teaching Hospital (UMTH), Yerwa Clinic (YC), and Bolori Clinic (BC), Maiduguri were recruited for the study. The SH and YC represent all-purpose hospital center with the

vaccinees mainly from the lower and middle socio-economic classes of the population. However, UMTH represent an institution-based health center with the vaccinees cutting across the middle and upper socio-economic classes. BC represents polyclinic with vaccinees also cutting across the middle and the upper classes of the population.

VACCINES:

Measles vaccines for a day's vaccination exercise from three tier of immunization centers namely Epidemiological Unit (Epid unit) with the storage facility of all vaccines allocated for Borno state, University of Maiduguri Teaching Hospital (UMTH) representing the Tertiary, Specialist Hospital (SP) representing the Secondary and Yerwa Clinic representing primary Immunization centers. In the course of collection of the vaccines, cold chain maintenance was also monitored at different period of the day .The management; storage and handling of the vaccines in each center were monitored by observation, questioning and counseling where necessary. Also aliquots of reconstituted and vials of unreconstituted measles vaccines were collected in few cases. The vaccines were transported in a vaccine carrier box to the WHO National Polio laboratory where they were stored at -20^0C until tested.

SERA:

One hundred and thirty-six children (aged 9-12 months) were bled for measles pre-vaccination sera. Of these 136 children, sera were also taken from 70 of the vaccinees'mothers. Four weeks later, 100 of the same children were bled for post vaccination sera. Serum samples were collected by finger prick method in Rapocca filter paper,) Rochester,Ml,USA) , dried at ambient temperature and stored in plastic bags at – 20^0C . Sera were extracted from the filter paper as previously described by Nakano et al (1983). All sera were heat inactivated at 56^0C for 30 minutes, treated with 25% kaolin to remove non-specific inhibitor and was absorbed with 50% monkey red blood cells (RBC) to remove non-specific agglutinins.

HAEMAGGLUTINATION TEST (HA) and HAEMAGGLUTINATION INHIBITION (HI) TEST:

The measles antigens used in the test were supplied by Dr Yoshi, JICA in Collaboration with World Health Organization (WHO) during a training course at Nogushi Memorial Medical Research Institute, Ghana 2001. The HA and HI test were carried out using WHO standard method and as described by Munube, 1979

VACCINE TITRATION:

The vaccines were titrated as previously described by Onoja et al 1992. The vaccine titer was calculated by the method of Reed and Muench (1938).

VACCINEES' PERSONAL DATA:Such data was based on questionnaire survey. The information collected from the Vaccinees include name, date of birth, sex, vaccination history, history of measles before the study if any and the age it occurred. Identification of the source of measles infection was also obtained (i.e. as an index case which is the first case in a household, or as a secondary case which is a child who develops measles between 6 to 20 days after the index case and under the same roof.)

3. RESULTS

Table 1 shows the HI antibody in children pre and post measles vaccination. Out of 236 children tested, 26 (16.9%) and 22 (23%) had measles HI antibody with GMT of 36.4 and 22.9 pre and post measles vaccination respectively. In table 2, measles HI antibody profile in mother-child pair is presented. Of 70 mothers tested, only 10 had HI measles antibody with GMT 34.3. Five mothers and their corresponding children had measles antibody with the GMT of 21.1 (mothers) and 9.2 (children pre vaccination) respectively. Also, five seropositive mothers (GMT= 55.7) had seronegative children. Nevertheless, 11 of 60 (18.3%) seronegative mothers had seropositive children with GMT 14.1 The HI antibody between mother and child (pre-vaccination) was not significantly different (Kw> 3.000 df. 2, P= 0.022313). In table 4 the potency of measles vaccines collected at different Immunization centers in Maiduguri Metropolitan is presented. Although varied titers of measles vaccines, (ranging from $10^{1.5}$ to $10^{6.0)}$ were observed in all the centers chosen for the study but they were not significantly different ($P > 0.05$).

The responses of vaccinees' mothers on clinical measles among Vaccinees and other children in the family:- Ten children (14.3%) had clinical measles infections before they were brought for vaccination at the age of 9 months. In addition 13 (18.6%) had index cases of measles in their families. No significant

difference was observed between measles antibody (pre-vaccination) and previous clinical measles infection (t= 0.23730, df. 24, P= 0814519).

Sex distribution of measles HI antibody:

Out of 134 males tested, 28 (20.9%) and 20 (19.6%) of 102 females had measles HI antibody Pre and post vaccination and the sex of the vaccinees were not significantly different from the measles antibody. Age at which measles occurred in index cases: The age at which index cases occurred in the family was significantly different from the titer of HI measles antibody pre-vaccination (F= 623432.600, df 4, P=0.00002).

Table 1. Measles Hi Antibody In Children Pre And Post Vaccination

Vaccination status	NO. tested	No. positive	Titres								
			4	8	16	32	64	128	256	1024	mean titre
Pre – vaccination	136	24 (17.6)	-	4	9	1	4	2	-	4	371.3
Post – vaccination	100	24 (24)	1	1	9	6	1	5	-	-	44.0
Total	236	48 (20.3)	1	5	18	7	5	7	-	4	415.3

Table 2. Measles Hi Antibody Profile In Mother – Child Pair.

S/NO	ITEM	MOTHERS (GMT)	CHILD	
			PRE- VACCINATION (GMT)	POST VACCINATION (GMT)
1	No. tested	70	70	50
2	No positive	10 (34.3)	10 (36.8)	16 (11.8)
3	No positive mother/ positive children	5 (21.1)	5 (9.2)	5 (9.2)
4	No positive mother/ negative children	5 (55.7)	0 (0.0)	0 (0.0)
5	No positive children/ negative mother	0 (0.0)	5 (12.1)	11 (14.1)

Table 5: The Potency Of Measles Vaccines At Different Vaccination Centres In Maiduguri

S/No	DATE OF COLLECTION/ DATE OF TITRATION	SOURCE (VACCINATION CENTRES)	BATCH NO.	EXPIRATION DATE	MANUFACTURER	TITRE ($TCID_{50}$/DOSE)
1	21-09-01/26-09-01	EPU a	1690	JULY 2002	SERUM INSTITUTE OF INDIA	$10^{3.2}$
2	17-10-01/17-10-01	EPU	1686	,,	,,	$10^{2.0}$
3	5-12-01/5-12-01	EPU	1680	,,	,,	$10^{3.0}$
4	5-02-02/5-02-02	EPU	EU1866	,,	,,	$10^{2.8}$
5	12-10-01/ 12-10-01	UMTH b	EU1688	,,	,,	$10^{2.0}$
6	7-12-01/7-12-01	UMTH	1680	,,	,,	$10^{3.0}$
7	5-02-02/5-02-02	UMTH	1683	,,	,,	$10^{2.8}$
8	12-03-02/12-03-02	UMTH	1683	,,	,,	$10^{1.8}$
9	22-05-02/22-05-02	EPU	EU1866	May 2003	,,	$10^{3.0}$
10	26-06-02/26- 06-02	EPU	EU1863	May 2003	,,	$10^{2.8}$
11	5-02-02/5-02-02	YERWA c	1676	,,	,,	$10^{2.8}$
12	22-05-02/22-05-02	YERWA	EU1866	May 2003	,,	$10^{1.5}$
13	26-06-02/26- 06-02	YERWA	EU1863	May 2003	,,	$10^{2.8}$
14	18-02-02/18-02-02	SP d	1676	,,	,,	$10^{2.8}$
15	22-05-02/22-05-02	SP	EU1866	2003	,,	$10^{3.2}$

a = Epidemiological Unit, Borno State. (Storage). b = University of Maiduguri Teaching Hospital, Borno State. (Tertiary)

c = A Primary Health Centre in Borno State (Primary). d = Specialist Hospital (General Hospital, Borno State.) (Secondary

4. DISCUSSION

Failure to deliver at least one dose of measles vaccine to all infants aged 9 months, is attributed as the primary reason for this preventable morbidity and mortality. However, the issue to be concerned with should include, how many of the vaccinated children are actually immunized bearing in mind the poor conditions of storage and transportation of measles vaccines to vaccination centers in Africa with particular reference to Nigeria.

The findings of this study show high degree of primary vaccine failure (76%) with very low (12%) rate of seroconversion. It was also observed that pre-existing measles HI antibody (with GMT 36.6) grossly interfered with the vaccine, resulting in low post vaccination GMT of 22.9. The low sero-conversion rate could be multicausal as demonstrated by Onoja et al (1992), to include subpotency of the vaccines, improper handling of the vaccines during vaccination, storage of the vaccines etc. In this study it was observed that health workers in different Immunization centers improperly handled vaccines during vaccination. For instance most frozen ice packs used for keeping the vaccine at the beginning of a day's vaccination exercise were not changed even when they became hot later in the course of vaccination. In addition, it was observed most often that, the vaccine diluent was not stored at the same temperature as the vaccine before reconstitution. Such malpractice could adversely affect the potency of the vaccine. The corroboration of the finding of Onoja et al (1992) with this current study implies that there has been little or no improvement in the handling of measles vaccines in vaccination centres. Therefore adequate monitoring of vaccination exercise in each center should be enforced.

This study revealed that 85.7% of the mothers of the vaccinees tested had no measles antibody at the time of measles vaccination for their children. Probably not all the 16.9% of 136 vaccinees with pre-vaccination measles HI antibody were of maternal origin but due to natural infections. This speculation was supported by the fact that ten mothers whose children had pre existing measles antibody admitted that their children contracted clinical measles before they were brought for vaccination at the age of 9 months. The lack of maternal antibody among the majority of the vaccinees' mothers probably explains why many children in Northeastern Nigeria usually contract and occasionally die due to measles before the age of 9 months (the recommended age for measles vaccination in Nigeria). Contrary to this report, 94.25% of children at age 9-11 years in Southwestern Nigeria had no pre existing measles antibody (Onoja et al, 1992) and this probably explains the high rate of seroconversion recorded in that study. In this report about 78% of the vaccinated children were still found to be seronegative. The implication of this is that cohorts of susceptible children are at the risk of contracting and causing measles outbreak in their respective communities at the slightest exposure to measles virus. In our study, 5 mother-child pair had measles antibody at the age of 9 months while children of five seropositive mothers had none. But whether the antibody in the former group was of maternal origin is still uncertain. However, it is expected that women at child bearing age should have been exposed to subclinical measles at various stagse of life. Therefore, the low maternal antibody among the vaccinees' mothers observed in this study needs further evaluation. Nevertheless, a study revealed that while 58% of Nigerian children lost their protective maternal antibody by the age of 4 months only 3% had enough antibodies to protect them between the ages of 6-9 months (Oyedele et al 2005). It is worth noting that these samples were collected in 2001/2002 when morbidity and mortality due to measles in Nigeria as a whole were very high. A report showed that, an overwhelming majority of the 561 deaths due to measles occurred in Northern part of Nigeria in 1999 (IRIN 2005). The high number of seronegative mothers and unimmunized children revealed in this study probably accounted for the menace caused by measles virus in northern Nigeria. In view of the observation in this study, when compared with that of Onoja et al, (1992), a parallel study of mother-child survey for measles antibody in both southern and northern Nigeria to ascertain the proper age at vaccination in Nigeria is suggested. Ensuring the quality of vaccines in terms of potency and cold-chain maintenance is very vital to the success of the Global measles vaccination Programme.

ACKNOWLEDGEMENTS

We wish to thank University of Maiduguri for supporting this project with a grant. Also, the technical assistance of David Bukbuk and management of UMTH is highly appreciated. We sincerely thank JICA for supplying the measles antigen through Dr Joshi

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Received: 8/2/2007

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Morphological And Leaf Epidermal features of Capsicum Annum and Capsicum frutescens solanaceae.

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ABTRACT: Studies on the morphological (vegetative and floral) and leaf epidermal features of Capsicum annum and Capsicum frutescens found in different parts of Imo State were investigated. This was with the main aim of evaluating their reliability as aid, in determining intraspecific relationship among these taxa. Morphological features among the two taxa studied include variation and in similarities, habit. The study show annual herb in *capsicum annum* and perennial herb in *capsicum frutescens*. Similarly the height of capsicum annum is 60cm, while *capsicum frutescens* is 72cm. Furthermore, the two taxa share common attributes as revealed in the stem, leaf and the floral morphology. The stem type, colour and bark shows that the two taxa are erect-branched from base, green and smooth respectively. The floral morphology furthers strengthens the Intraspecific relationship among these two taxa. The floral result reveals that the flower type, symmetry, Arrangement, Pedical Calyx and corolla colour and shapes are all the same in the two taxa studied. The leaf epidermal characters in the two taxa studied did not show much variation except in the number of stomata: 21 and 44 and 18 and 60 on he upper and lower surfaces of *capsicum annum* and *capsicum frutescens* respectively. The other attributes of stomata type, number of subsidiary cells, shape of epidermal cells and presence of trichome are the same for the two taxa hence this study confirms the values of morphological and leaf epidermal features in the systematics and biological consideration of *capsicum annum* and *capsicum frutescens.* [Nature and Science. 2007;5(3):54-60]. (ISSN: 1545-0740).

Keywords: Morphology; Leaf epidermis; Capsicum; Solanaceae; Systematics

INTRODUCTION

The genus *capsicum* belongs to the family solanaceae (Night shade). Members of the solanaceae family are mostly herbs or under herbs while some others are climbers. The family contains about 90 genera and nearly 3000 species (Vidyarth and Tripatha 2002, Stern 2000). The genus *capsicum* is further classified into the division Magnoliophyta, class magnoliopsida, order solanates and family solanaceae (Heiser and Smith 1953). *Capsicum* is a crop that is widely cultivated because of its spicy nature and nutritional value. The crop accounts for a large portion of vitamins A and C in many Nigerian diets. *Capsicum annum* and *capsicum frutescens* are the most common species in Nigeria. Heiser and Smith (1953) distinguished two *capsicum* species cultivated as vegetables while varieties are all froms of either *capsicum annum* or *capsicum frutescens*. *Capsicum annum* is not known in a wild state and species commonly cultivated are *capsicum annum* known as sweet pepper, bell pepper, cherry pepper and green pepper (Messraen 1992). *Capsicum frutescens* on the other hand occurs in the wild though became domesticated in many parts of the tropics. Species commonly cultivated is *capsicum frutescens* are known as bird eye pepper, red pepper and Tobasco pepper (Heiser and Smith 1953). Their economic importance has been discussed. In West Africa and in Nigeria in particular, *capsicum annum* and *capsicum frutescens* are third among the cultivated vegetables being utilized in the dry state as spice, *capsicum* content, an alkaloid that is a digestive stimulant is used in ointment for leaf of arthritic and neuropathic pains (Uzo 1982, Stern 2000). *Capsicum* species are rich in Vitamin A potency which is responsible for red colour in mature fruit, as well as ab out 50 - 280 Mg/100g of Vitamin C. *Capsicum annum* and *capsicum frutescens* are further used as pungent spices for domestic culinary purposes and by food manufacturing industries for seasoning of processed foods in the preparation of curry powder, hot sauce and in pickling (Tindall 1986). *Capsicum* species are mostly herbs with branched top roots. The stem is herbaceous, erect and hairy; leaves are alternate, opposite in flora region, simple and estipulate. The placentation is axile, ovules are numerous, style single terminating in a bilabed stigma. The fruit is a berry and the seed are minute endospermic with a straight or curved embryo (Esula 1977). The use of morphological and leaf epidermal features has been found to be of immense interest in taxonomy. An excellent review of the application of morphological features in systematic studies is shown in the works of Okwulehi and Okoli, 1999, Chakrabarty and Gupta, 1981 and Olowokudejo, 1990: Edeoga and Eboka 2000, Edeoga and ikem 2001 and Stern 2000.

Furthermore, the use of leaf epidermal features (epidermal cell, stomata and trachoma) in systematics has become popular and distinctive and have been used as a great taxonomic tool at the levels of family, genus and species. The works of Paliwal (1967), Shah and Gopal (1972). Gill Karatela (1982), Edeoga (1991) Edeoga and Osawe (1996) are typical examples.

This study assesses the relevance of morphological and leaf epidermal features of *capsicum annum* and *capsicum frutescens* as well as to evaluate the reliability of these characters in the systematic consideration of the *capsicum* species studied.

MATERIALS AND METHODS

The laboratory was carried out at the plant Science and Biotechnology Department of University of Nigeria Nsukka between September and November, 2006. These studies were made on mature living fresh materials of *capsicum annum* and *capsicum frutescens* collected from the garden behind the Education trust fund Block 11, the staff nursery/primary school garden and the garden beside Saint Joseph Chaplaincy all within Imo State University, Owerri, Imo State.

For Moephological studies Twenty mature leaves of each taxa from the middle portion of the plants were collected length and width of the leaves were measured using a zoom meter rule. The length of the leaf was obtained by spreading the middle leaflet on a flat surface on the laboratory bench, while for the width the same media leaflet was chosen and measured to ensure uniformity Olowokudgo (1990). The seed number per pod was obtained by counting the number of seeds in the biggest pod of each taxa to ensure consistency. Photographs of the fresh materials were taking using ordinary camera and characters of the two taxa were divided into vegetative and floral morphology and tabulated.

For the leaf epidermal features, twenty samples of each species of *capsicum* studied were examined using light microscope. An area of about 1cm square was removed from a central standard position, always midway between the base and Apex of the mature and fresh leaves of the two taxa studied. Epidermal preparations were made by boiling the collected materials of each of two taxa in different test tubes containing 70% ethanol for 10min. These were allowed to cool and latter bleached in 8% sodium hydrochlorite (Naocl) for 5min.

Epidermal peals were stained with 1% ethanol safranin and temporarily mounted in aqueous glycerol solution (Cutler, 1978). Photo micrographs of the epidermal features were taken from the slides using a Leitz wetzlar ortholux microscope fitted with a vivitar v-35 camera.

RESULTS

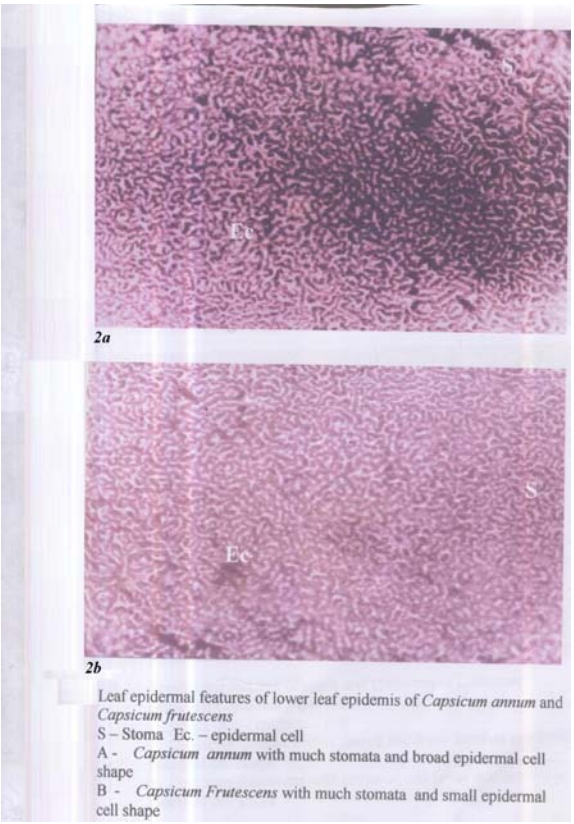
The morphological (vegetative and floral) and leaf epidermal features of *capsicum annum* and *capsicum frutescens* investigated are summarized in Table 1, 2 and 3 for the morphology and Table $ for the leaf epidermis and illustrated in (Fig. 1a – b and 2a – b). The vegetative results of the two taxa studied showed that the habit and height of *capsicum annum* annual herb and 60cm while of the habit and height of *capsicum frutescens* is perennial herb and 72cm respectively. The habit still can from the herbecous nature of these taxa. The attribute of stem type, colour and bark are similar to both taxa. Similarly the leaf shape, leaf Apex, leaf base and leaf type equally revels similarity in both taxa except for leaf arrangement which is Alternate in *capsicum annum* and opposite in *capsicum frutescens* Table 2.

Furthermore, the floral result of the two taxa studied showed that the attribute of flower type, floral symmetry, pedicel, calyx colour, shape, corolla colour, fruit type are the same in the taxa. The difference in the floral result is observed in the flower arrangement: opposite in capsicum annum and Alternate in *capsicum* frutescens,: Corolla fusion: fused in *capsicum annum* and free in *capsicum frutescens* and fruit shape indicates ovoid in *capsicum annum* and linear in *capsicum frutescens* Table 3.

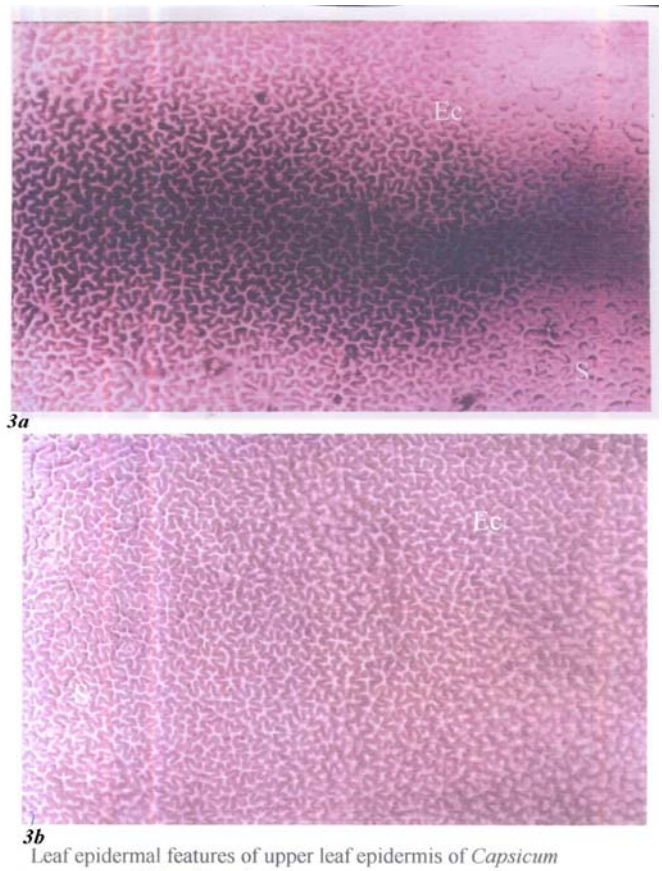
The result of the leaf epidermal features of *capsicum annum* and *capsicum frutescens* investigated are summarized in Table 4. The shape of the epidermal cells of the upper and lower surfaces is irregular and sinuous in both taxa studied. The two capsicum taxa were amphistomatic having stomata at both the adaxial (upper) and abaxial (lower) surface of the leaf. The distribution of stomata on both the upper and lower surface of the studied reveal anomocytic type of stomatal arrangement. The stomatal index range from 18% - 21% in the upper surface of two taxa studied respectively and from 44% in *capsicum annum*, 60% in *capsicum frutescens* in the lower surface respectively. This implies that the stomatal density is therefore highest on the lower epidermis and lowest on the upper epidermis. They were absence of trochomes in the *capsicum* taxa investigated.

Fig 1a: Habit of Capsicum anum

Fig 1b: Habit of Capsicum frutescens



Leaf epidermal features of lower leaf epidemis of *Capsicum annum* and *Capsicum frutescens*
S – Stoma Ec. – epidermal cell
A - *Capsicum annum* with much stomata and broad epidermal cell shape
B - *Capsicum Frutescens* with much stomata and small epidermal cell shape



Leaf epidermal features of upper leaf epidermis of *Capsicum annum* and *Capsicum frutescens*
S – Stoma E.c – epidermal cell
A – *Capsicum annum* with scattered and fewer stomata and broad epidermal cell shape
B – *Capsicum frutescens* with scattered and fewer stomata and small epidermal cell shape

Table 1. Vegetative characters of the two Capsicum species studied

Character	*Capsicum annum*	*Capsicum fructescens*
Habit	Annual herb	Perennial herb
Height	60cm	72cm
Stem type	Erect branched from base	Erect branched from base
Colour	Green	Green
Bark	Smooth	Smooth
Leaf texture	Smooth	Smooth
Leaf arrangement	Alternate	Opposite
Leaf shape	Ovate	Ovate
Leaf apex	Mucronate	Mucronate
Leaf base	Round	Round
Leaf type	Simple	Simple
Length(cm)	9.9± 1.13	5.35±0.15
Width (cm)	4.9±0.8	4.2±1.1

Table 2. Floral morphological characters of the two Capsicum species studied

Character	*Capsicum annum*	*Capsicum fructescens*
Flower type	Auxillary cyme	Auxillary cyme
Floral symmetry	Actinomorphic	Actinomorphic
Arrangement	Opposite	Alternate
Pedicel	Erect	Erect
Calyx colour	Greenish white	Greenish white
Shape	Elliptic	Elliptic
Corolla colour	White	White
Corolla free/fused	Fused	Free
Fruit type	Pod	Pod
Fruit shape	Ovoid	Linear
Seed number per pod	4 seeded	6-8 seeded
Pod length(cm)	1.5±0.11	5.35±0.15
Pod width(cm)	2.65±0.25	4.15±0.35

Table 3. list of *Capsicum* species from which fresh materials were collected

Collection number	*Taxa*	*Locality*	*Collector(s)*	*Place of deposition*
001	*C .annum*	Staff nursery/primary school garden Garden behind education trust fund block ‖ Garden beside St Joseph catholic chaplaincy	Onyeji Augustina Nwamaka Ojimba Chioma Akpaka Doris	IMSUH
002	*C. fructescens*	Staff nursery/primary school garden Garden behind education trust fund block ‖ Garden beside St Joseph catholic chaplaincy	Iwuchukwu Ikechukwu Iwu Jane Ochiji Chidimma	IMSUH

IMSUH: Imo State University Herbarium

Table 4. Epidermal characteristics of the Capsicum species studied

Characters	*C .annum*		*C .fructescens*	
	Upper surface	Lower surface	Upper surface	Lower surface
Shape of epidermal cell	Irregular and sinuous	Irregular and sinuous	Irregular and sinuous	Irregular and sinuous
Stomatal type	Anomocytic	Anomocytic	Anomocytic	Anomocytic
% stomatal index	21	60	18	44
Number of subsidiary cells	None	None	None	None
Trichomes	Absent	Absent	Absent	Absent

DISCUSSION

The result of the morphological and leaf epidermal features of *capsicum annum* and *capsicum frutescens* studied show some characteristic that could be used for taxonomic decision. Morphologically, the vegetative features of habit and height of *capsicum annum* separate it from *capsicum frutescens*. The observation is in line with earlier works of Okwulehi and Okoli, 1999, and Edeoga and Eboka 2000, who used comparative morphology of different species in establishing relation among various taxa. The result of leaf arrangement showed alternate shape in *capsicum annum* and opposite in *capsicum frutescens*. This observation is supported by the works of Edeoga and Eboka 2000, in Dissotis (Okeke and Nwachukwu 2001) in Euphorbiaceae but not in the genus *capsicum*.

The result of the leaf epidermal studied indicates that the shape of epidermal cells of the *capsicum* species studied are irregular and sinuous. Similarly, the two taxa are amphistomatic with more stomata on the lower surface (abaxial) than the upper surface (adaxial). The percentage stomatal index of the two taxa was highest on the lower epidermis 44% – 60% compared to the epidermis and 18% – 21%. The stomatal index result is not strange since Olowokudejo (1990) found stomatal index valuable and very reliable indistinguishing between the leaves of medicinal species of *ocimum* from non medicinal ones. The absence of irichome in the two taxa studied is not of taxonomic importance since Esua (1977) insisted that much reliability is not always accorded to trichomes alone for taxonomic conclusion due their similarity in different species. The anomocytic type of stomata found in the two *capsicum* species indicates that the species are phylogenetically related (Mbagwu 2005). The morphological (vegetative and floral) and leaf epidermal features of the taxa studied conforms the intraspecific relationship between *capsicum annum* and *capsicum frutescens* in their stem, leaf, flower, fruit, seed, stomatal type and index attributes hence the differences in leaf and flower arrangement, fusion of corolla and fruit shape are not enough to separate the two taxa studied. These distinguishing morphological and leaf epidermal features observed in this investigations are of systematic value because they are reasonably constant in the taxa stuied. Olowokudejo (1990) made similar observation in the genus *Anonna*. The purpose of this study is to show that application of morphological features has proven to be of immense assistance in interpreting problems related to pant classification. Thus the necessity of including the results from the morphological and leaf epidermis with data derived from other botanical disciplines remain vital when formulating conclusions on the systematic of the *capsicum* species.

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Received: 9/14/2007

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Body Mold Setting and Motion Emulation Analysis of Steering Mechanism of Two Power Flow Tracked Vehicle based on CATIA

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Abstract: CATIA is developed by Dassault System of French, one of integration Software of CAD / CAE / CAM. 3-D model building-up process of control body and part has been introduced detailedly using CATIA in the paper, tracked vehicles adopted double power flow differential turning mechanism, and carried out suppositional assembling. Validated characteristic and ruled through movement emulation. On designing the software platform in CATIA 3-D, electron model machine technology has been emulated in DMU Kinematics under Digital Mockup. Interference detection has been carried on whole device considering interference phenomenon between parts in motion. [Nature and Science. 2007;5(3):61-66]. (ISSN: 1545-0740).

Key words: CATIA；tracked vehicle；two power flow steering；setting mold；motion emulation

1. Introduction

CATIA is developed by Dessault System of French, one of integration Software of CAD / CAE / CAM. 3-D model building-up process of control body. CATIA has pieces of 11-modules and Including infrastructure, mechanical design, shape, analysis and simulation, AEC factories, NC models, equipment and systems, the processing of digital processes, ergonomic design and analysis, and so on. CATIA introduce feature modeling and parametric modeling technology that allows automatic or designated by the user-specific design parameters, or function of the geometric constraints of the design variables. The assembly design module based on the establishment and management of parts and 3D mechanical restraint installed accessories, auto parts on the connection between the definition and facilitate the movement mechanism for early analysis, greatly accelerated the purchase of accessories design, the follow-up application can use this model to the design, sub - Analysis and manufacturing. DMU module assembly process to be completed by the Movement for the establishment, analysis and interference checking, and the path planning and spatial analysis. According to its line of 3D planes, users can accurately the establishment, modification and analysis of 3D geometric models.

CATIA is a feature-based parametric solid modeling system, the traditional CAD technology for the fixed size of the geometric definition of the value elements of each input line has identified a location and length, in order to amend the map's content, only to delete the original lines and anew painting. While the development of new products need to repeatedly design changes，that make the shape and size of components to the comprehensive coordination and optimization. Stereotypes for product design, we need to create products, in order to address the production features users with different types of products. Parametric Design will design products with certain structural changes and the size automatically modified graphics. Feature-based design is the design of products as the basic unit and mechanical products described as an organic combination of characteristics.

2. Control components 3D model of building-up

Doing virtual assembly and motion simulation before, we must establish a system composed of parts of 3D models. This set of manipulating 3D modeling agency of this process, the control panel of tilt the modeling as an example, According to the first parts of the structure, the use of CATIA software module mechanical design of sub-module -- Part Design module functions entering into sketching interface, rendering the tilt control panel of wire frame, as shown in Figure 1.

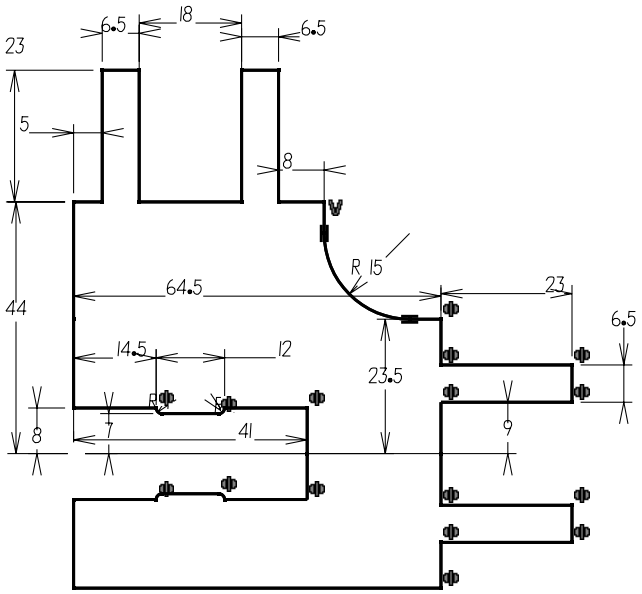

Figure 1. The sketch of controlling plate

Then parts of the functional module design, drawing, rotation, scanning and grooving, drilling, a range of features such as modeling, tilt control panel completed the three-dimensional modeling, as shown in figure 2.

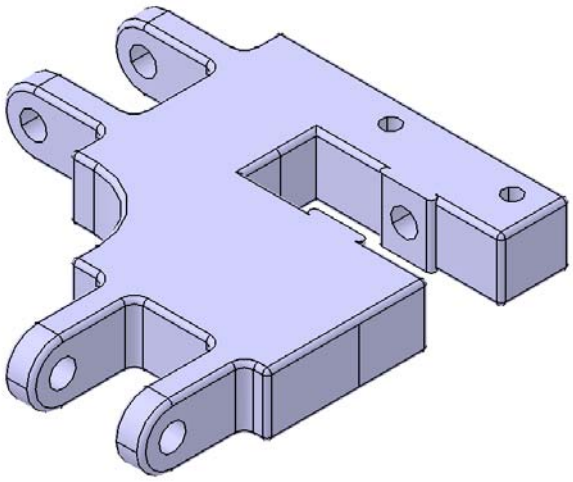

Figure 2. The entity model of controlling plate

Other parts of the modeling using the same steps, the first parts for sketching, and then use the function module parts, the parts were for 3D modeling, model other parts, as shown in Figure 3 typical Parts explosion map, thus to complete all parts of 3D solid modeling.

3. Virtual Assembly

Assemblies of many parts and sub-assembly of organic composition, which is the expression of the two-part information, part of the entity information is assembling the body parts of the combined entity information; Another part is assembling information intimate parts of the interrelationship between information. CATIA 3D software such as providing a powerful virtual assembly functions. Assembly body design module provides a powerful virtual assembly parts function. In this module, through parts of coaxial

elements, coplanar, distance, angle, as well as the anchor bound to achieve the virtual assembly parts. According parts entity model, the mechanical design module module -- assembly design (2935D Design) module, the import of spare parts assembled model for the appropriate location on the adjustment would make it easier to impose restraints. Then, using the toolbar binding orders, location binding methods used to achieve assembly constraint modeling, to ensure proper parts in parallel, coaxial or coplanar relationship. Construction Control Box typical parts entity model shown in Figure 3.

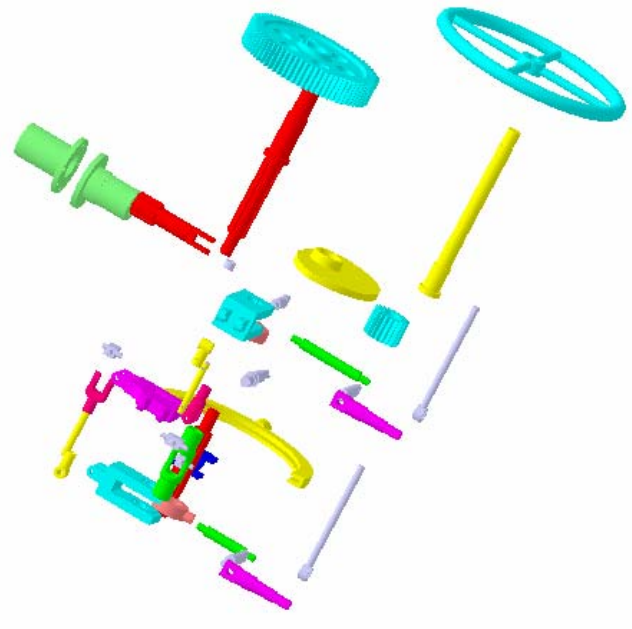

Figure 3. Entity model of typical parts

After the assembly of products also use the assembly analysis functions, the assembly of products for various analytical relations. If there is interference between parts happen, it could directly on the platform assembly of parts changes, so that only the actual assembly of the parts can be found at the series of problems now in modeling design to discover and remove

4. Motion Simulation

Motion simulation in the design of direct digital model （Digital Mockup） under the DMU Kinematics （digital simulation module）.Enter the sub-module DMU Kinematics, by the introduction of assembly design model. Then, using tools Kinematics Joints of the various campaigns deputy sports relations between the definition, sports institutions, the connection is generally a rotary joint （Revolute Joint）, sliding joints （Prismatic Joint）, cylindrical joints （Cylindrical Joint）, screw joints（Screw Joint）, and other connection methods, the use of simulation modules with orders （Simulation with commands） simulation can be achieved satisfactory results. As shown in figure 4.

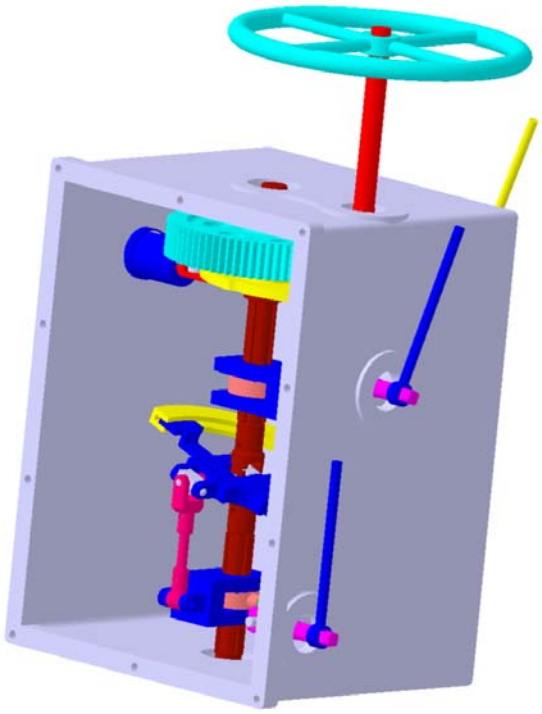

Figure 4. The simulated diagram of the mechanism while forward right steering situation

From Figure 4 can be manipulated to see a moving shot put with the rotation of the steering wheel and a swinging angle of this vehicle to achieve progress right turn, at the same time manipulating the straight shot put will gradually return to the initial position that will control line separating the speed gradually reduced, the deceleration of vehicles, the biggest point of the steering wheel to 360 ° manipulating straight shot put will return to the initial position and realize vehicle to the right place.

5. Interference Detection

Interference checking including static interference checking and dynamic interference checking, static interference checking refers to the virtual assembly structure, assembly inspection of the body parts between the relative position between the existence of interference, the assembly tolerance design is reasonable; Dynamic interference checking and assembly of the parts off the assembly campaign Cheng, his campaign enveloping body parts between the existence of a campaign to interfere. This sets manipulation detection devices to interfere in the process, the main consideration in this set of campaign process, the availability of components between interference, if it exists, the agency inspected to ascertain first assembly of parts between connecting whether there was a problem, if there should re-form its connections into OK bound connections, connecting to determine the form only after further interference detection, if there is still interference, we should interfere parts inspection, the right to re-amend parts, the number of specific changes, according to the test results interfere in the quantity to decide. The final revised the dynamic interference test results shown in Figure 5.

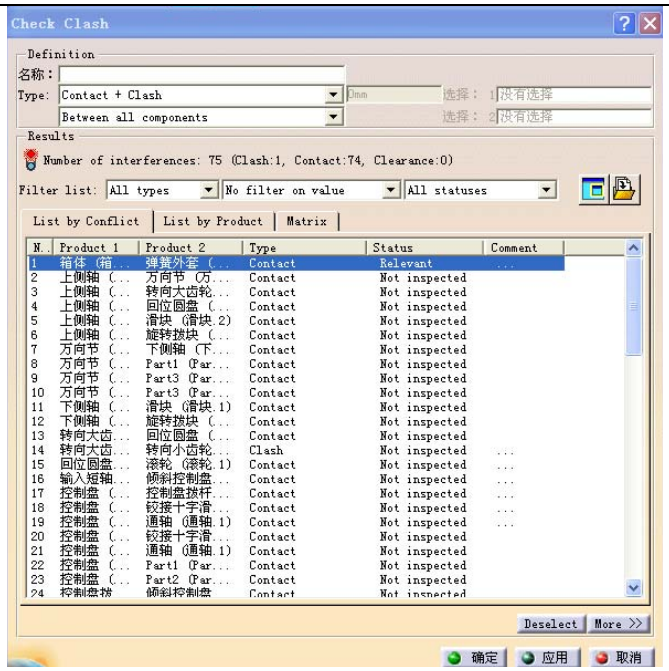

Figure 5. The result of interference analyze

Completion of this step can further three-dimensional map of the output of two-dimensional drawings, graphic drawings of simple changes can be conducted on the production and processing. Thus, the application of this set of CATIA software control agencies design and analysis greatly shorten the design cycle, and guarantee quality of the design.

6. Conclusion

（1）described the use of CATIA dual-tracked vehicle power flow shifted control box components 3D model of the process, virtual assembly, motion simulation and analysis of this set of certification bodies and the movement characteristics of movement.

（2）CATIA 3D design software platform, electronic technology in the digital prototype model （Digital Mockup） under the DMU Kinematics （digital simulation module） for motion simulation, and consider this set of institutions in the process of movement between the parts availability interference, manipulation package for a device interference Detection.

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Received: 9/17/2007

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Synthesis and characterization of hybride polyaniline / polymethacrylic acid/ Fe_3O_4 nanocomposites

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Abstract: In this paper, we present the synthesis of Fe_3O_4 nano particles of hybrid for coating on metal or glass surfaces.The synthesis of the organic/inorganic nanocomposite is described. This nanocomposite is a water – dispersible polymeric complex of polymethacrylic acid and polyaniline.The water dispersible hybride material was coated on aluminum using a molecular layer – by – layer coating. The composites, Thus synthesized have been characterized by infrared spectroscopy and X-ray diffraction. The morphology of these samples was studied by scanning electron microscopy. [Nature and Science. 2007;5(3):67-71]. (ISSN: 1545-0740).

Keywords: Hybride nanocomposites, polyaniline , polymethacrylic acid

1. Introduction

Polymer nanocomposites constitute a class of hybrid materials composed of a polymer matrix and an inorganic component which has at least one dimention in the nanometer (<100 nm) size domain. Nanoparticles were first developed around 1970. The synthesis of metallic nanoparticles has become an extremely interesting topic in the field of material science , due to the wide range of optical and electronic properties that are accessible in the nanometer –size regime.Nanometer scale hybride materials is currently an area of active research.[1] These nanomaterials are hybride between organic and transition metals or rare earth oxides , for example cerium oxide , ferrous oxide ,… [2]. metallic nanocomposites have emerged as a new class of materials because of their unique electrical, optical and chemical properties[3].

The properties of a polymer – reinforced composite are mostly influenced by the size, shape,composition ,state of agglomeration, and degree of matrix inorganic component[4]. Decreasing the particle size to the nano-size dimension influence the macroscopic properties of the polymer because a breakdown of the common rule – of – mixture theory occurs[5]. This breakdown is caused by the amount of interfacial zone that gains importance with respect to the phase relative to bulk behavior[6].

Demonstrated application of such composites can be found ,among others, in the fields of optics, mechanics,iono-electronics,biosensors, flame retardants and membranes.

In this paper , we explore the synthesis of the new hybride material with the water- dispersible polyaniline /polymethacrylic acid as the organic component and the nano solution of Fe_3O_4 at the sodium dodecyl sulfate /H_2O as the inorganic component. The nano magnetic Fe_3O_4 has a diameter between 10 to 12 nm. We report the synthesis method of polyaniline and polymethacrylic acid nanocomposit with Fe_3O_4 nano particles. Since the organic / inorganic hybrid nanocomposites are dispersible in water, they are suitable for coating on glass or metal substrates.

2. Experimental

Materials

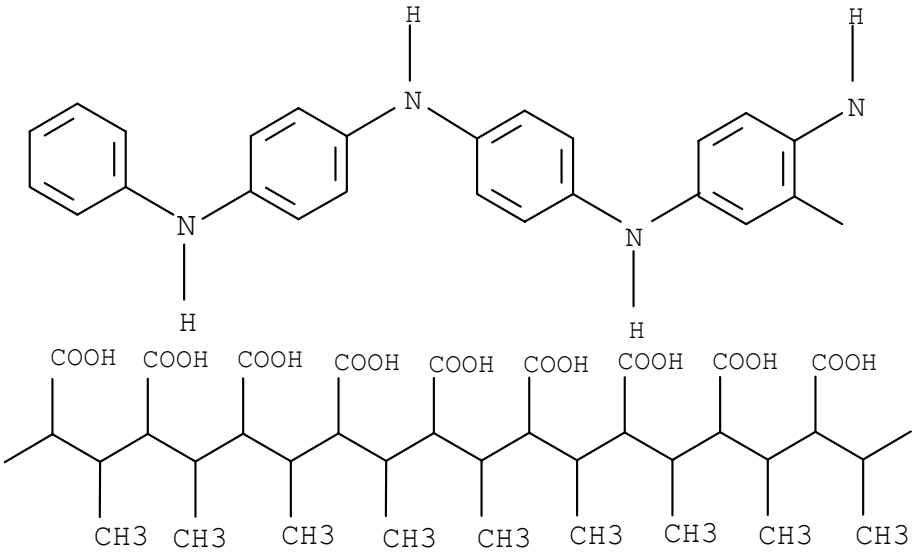
Nano particles of Fe_3O_4 were purchased from nanotechnology center of baku state university. The particles have an average of 10 - 12 nm. Methacrylic acid and aniline were purchased from Aldrich chemicals. Aniline was purified by distillation under vacuum.

Instruments

The images of nanoparticles were investigated using Philips XL30 scanning electron microscope. The Fourier transfer infrared (FTIR, Bruker) spectroscopy was used to identified the polymer on the Fe_3O_4 nano particles surface. Spectra were obtained in the wave number range of 400-4000 cm^{-1}. Spectra of the polyaniline / poly(methacrylic acid) modified Fe_3O_4 nanoparticles were recorded from KBr in 1:10 (wt/wt) ratio.

Synthesis of polyaniline: polymethacrylic acid complex

The organic component of this nanocomposite is an inter- polymer complex of conducting polymer[7] .Previously the method for synthesizing the molecular complexes PAN:PAA was reported[8]. This component consists of two polymer s that a double-strand molecular complex [9].The first strand is polyaniline (PAN) and second stand is polymethacrylic acid (PMAA).We use polymers PAN:PMAA as a short hand representation of the complex. Figure 1 show a structural representation for PAN:PMAA.



The several method for synthesizing the molecular complexes was previously reported [10] .This synthesis base involves two steps : In the first step , monomers of aniline are adsorbed onto the poly(methacrylic) backbone by molecular self –assembly. In the second step, the adsorbed aniline monomers are polymerized by an oxidant (potassium persulfate) to form polyaniline.

Adsorption of aniline on poly(methacrylic acid)

0.012 mole (3.5 gr) poly(methacrylic acid) (90,000 MW) dissolve in 25 grams distilled water. Stir for 2 hours. Then add 0.558 gram of aniline to solution, stir for 4 hours to allow equilibrium adsorption. The molar ratio of the PMAA component to the aniline is 2:1 .

Polymerization of aniline to form the polymer complex

Acidifiy the solutiom prepared by adding 4 ml 3M Nitric acid .Dissolve 0006 mol potassium persulfate with 10 ml distilled water. Mix these two solutions to start the polymerization reaction. The solution turned to dark green. Stir for 24 hours to obtain a homogeneous solution of the polymeric complex. The polymeric complex is a dispersion of particle of 100-200 nm in diameter. The infrared absorption spectra of the complex are consistent with the structure of a polymeric complex of polyaniline and poly(methacrylic acid) .

The physical absorption of the polymeric complex and nano Fe_3O_4 particles

Stir Fe_3O_4 dispersive solution with sodium dodecyl sulfate / distilled water with mechanical stirring. A diluted solution of the PMAA: PAN complex is then mixed with Fe_3O_4 solution (25% Fe_3O_4) for 10 minutes. The average molar ratio of the components is PMAA:PAN:Fe_3O_4 = 2:1:2. The dispersion is stable with very small amount of precipitation.

3. Results and Discussion

Scanning electron micrography

SEM of polyaniline – poly(methacrylic acid) nanocomposite synthesized by chemical oxidative is shown in Figure 1(P3-A). PAN/PMAA nanocomposite is very sensitive to the temperature. Due to the intractionelectron and sample. Scanning electron micrography images were obtains from a diluted solution of the nanocomposite particle. The white spots are Fe_3O_4 nano particles. The SEM image shows the presence of spherical Fe_3O_4 particles in PAN/PMAA composite, which are homogenenously distributed throughout the composites, which is also confirmed from XRD studies[11].

X-ray diffraction

The crystallinity of the formed composites was followed with X-Ray diffraction(XRD) as s function of weight percent inorganic component. Figure 2a shows X-ray diffraction pattern of polyaniline – poly(methacrylic acid) complex (0 % Fe_3O_4) .Diffraction of PAN-PMAA have a broad peak at about 2Θ = 25.92 ° , which is a characteristic peak of PAN-PMAA (Wan et al 1994 , Wan and li 1998) . Studies on XRD patterns of PAN-PMAA are scarce in the literature (Rajendra Prasad and Muunichandriah 2002). Figure 2b shows the XRD pattern for PAN-PMAA-Fe_3O_4 (25%). The diffraction pattern of PAN-PMAA-Fe_3O_4 nanocomposite shows a peak at about 2Θ = 26.89°[12] .

Fourier transfer Infrared spectra

Figure 3 shows the FT-IR spectrum of polyaniline – poly(methacrylic acid) nanocomposite , where the % of transmittance is plotted as a function of wave number (cm^{-1}). The characteristic FT-IR peak at 1523 and 1485 cm^{-1} are due to the presence of quinoid and benzenoid rings, respectively and are clear indication of these two states in the polymer chain. Also, The peaks at 1176, 1710 cm^{-1} are due to the C-N, C=O bond stretching vibration ,respectively . Figure 4 shows the FT-IR spectrum of polyaniline – poly(methacrylic acid) composite with Fe_3O_4 nanopaticles. The FTIR spectra of PAN/PMMA composite in presence of Fe_3O_4 exhibite new adsoption peaks distinctly at 1562,1479,1295,1132 and 799 cm^{-1} which are assignable to the presence of various metal oxide in the composite.

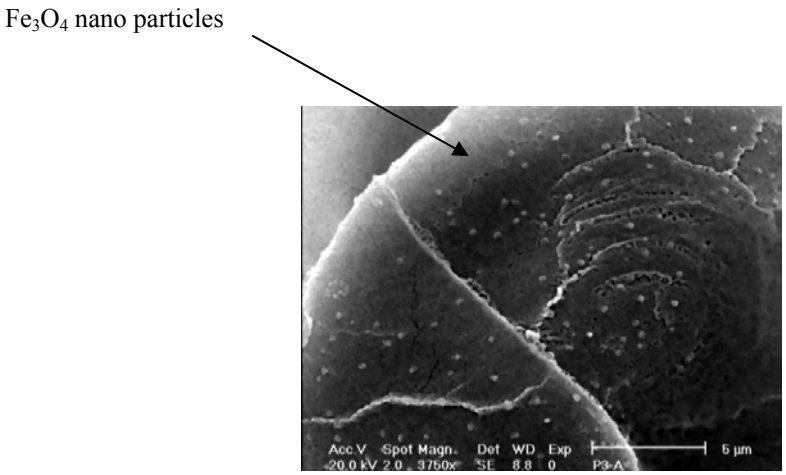


Figure 1. Scanning electron micrograph of PMAA:PAN:Fe_3O_4 nanocomposite

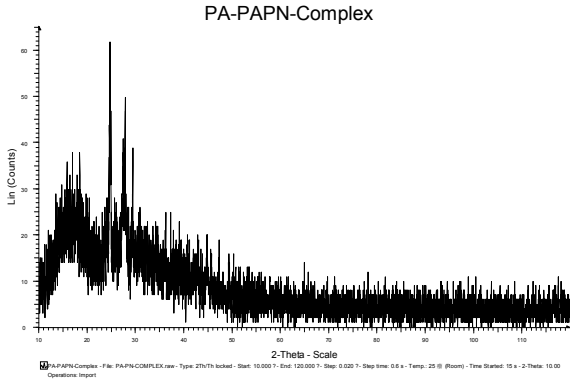


Figure2a. XRD spectra of polyaniline – poly(methcrylic acid) complex

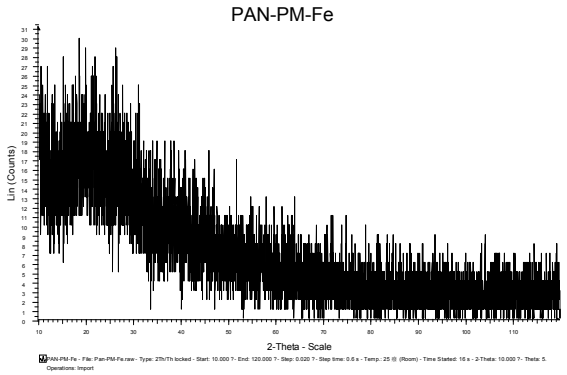


Figure 2b. XRD spectra of polyaniline/ poly(methcrylic acid)/Fe_3O_4 nanocomposit

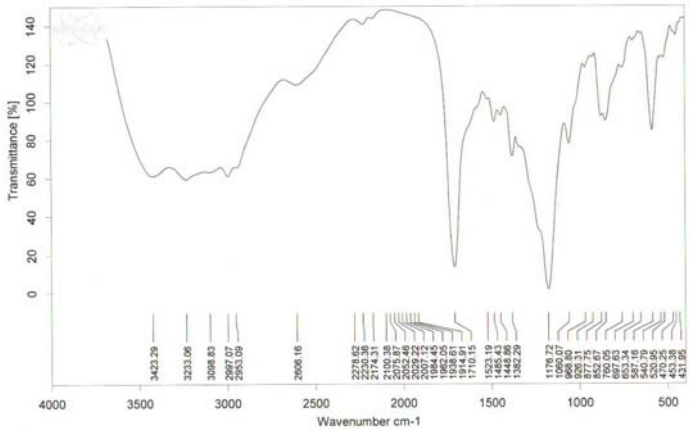


Figure 3. FT-IR spectra of polyaniline / poly(methacrylic acid) complex

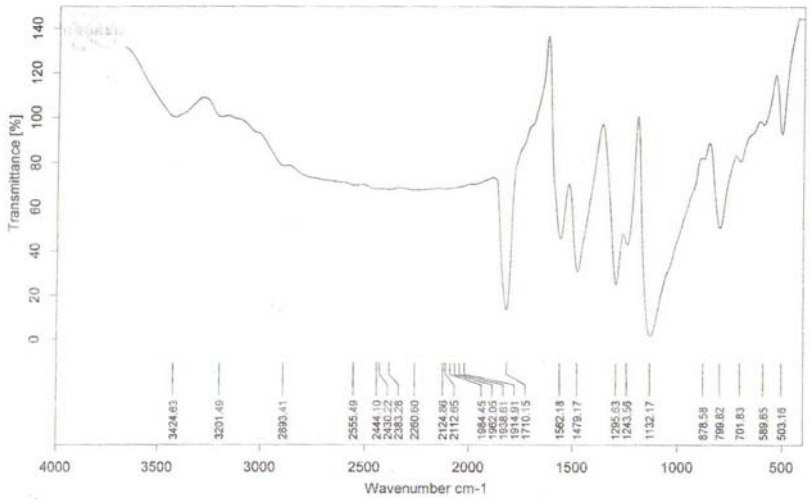


Figure 4. FT-IR spectra of polyaniline / poly(methacrylic acid) / Fe_3O_4 nanocomposite

4. Conclusions

We have synthesized new hybride polyaniline – poly(methacrylic acid) / Fe_3O_4 by in situ polymerization in the presence of Fe_3O_4 nano particles .This nanocomposite show semi-crystalline nature, whereas the PAN-PMAA synthezied is amorphous in nature. The SEM photograph of nanocomposite with 25% Fe_3O_4 show the presence of cenospheres.

These nanocomposites are suitable materials for high technology industries. The organic component is the hybride material have the dimention of 100-200 nm. One type of the composite is synthesized by preparing a precursor that contains the Fe_3O_4 nano particles, the poly(methacrylic acid) and the aniline monomers.

The composites were coated on glass and metal surfaces by the method of layer-by layer coating of self – assembled multi layers.

Acknowledgment

The authors Wish to thank M. Allahverdiev (Baku State University) for Valuable discussions.

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Ten Propositions for the Law of Increasing Marginal Utility

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Abstract: It is called "the law of diminishing marginal utility" that Marginal utilities are diminishing across the ranges relevant to decision-making. However, it will not always hold. Sometimes an amount added brings things past a desired "tipping point", or an amount subtracted causes them to fall short. In such cases, the marginal utility of a good or service might actually be increasing. This paper investigates Xinfei Corporation's rapid growth, and proposes ten propositions for the law of increasing marginal utility. [Nature and Science. 2007;5(3):72-77]. (ISSN: 1545-0740).

Keywords: Marginal Utility, Mechanism of Creative Process, Three-color Business Management

1. Introduction

Since 1986, Xinfei Corporation, a Chinese high-tech company, grows rapidly. In this paper, we compare the development of Xinfei Corporation with the development of other Chinese companies and some foreign companies, and conclude that: (1) Under China's new market economy mechanism, Xinfei developed in a new progressive mode, "Invest → Increase → Re-invest → Re-increase"; (2) The most important fact of Xinfei's development is "Innovation". These two conclusions can partially prove the law of increasing marginal utility. Thereby, management innovation can be constructed on this law. The law of increasing marginal utility provides a new economic theory for business management innovation. We also propose ten propositions for this law. These ten propositions play a significant role in economics, which can be considered as economic "Goldbach Conjectures".

2. The law of increasing marginal utility

2.1 Related work

In this paper, we focus on the proof and explanation for the law of increasing marginal utility based on the reality of Xifei's amazing development. Using this law, a new development mode for companies in the current era based on information economics is proposed as: "Innovation → More Innovation". This mode will result in a new revolution for companies' administration. Up to the present, the law of increasing marginal utility is still a state-of-arts theory.

In *The Manifesto of Communism* [1], K. Marx and F. Engels proposed that the bourgeoisie cannot exist without constantly revolutionizing the instruments of production, and thereby the relations of production, and with them the whole relations of society…The bourgeoisie, during its rule of scarce one hundred years, has created more massive and more colossal productive forces than have all preceding generations together.

In *Capitalism*, *Socialism and Democracy* [2], J. Schumpeter proposed that as soon as quality competition and sales effort are admitted into the sacred precincts of theory, the price variable is ousted

from its dominant position…But in capitalist reality as distinguished from its textbook picture, it is not that kind of competition which counts but the competition from the new commodity, the new technology, the new source of supply, the new type of organization (the largest-scale unit of control for instance) – competition which commands a decisive cost or quality advantage and which strikes not at the margins of the profits and the outputs of the existing firms but at their foundations and their very lives. This kind of competition is as much more effective than the other as a bombardment is in comparison with forcing a door…

The above propositions have explained why capitalist economy grew such rapidly, and how this economy machine ran automatically. In addition, they discussed how innovation plays an important role in the open market economy, and explained the open market economy by "innovation". Our research, the law of increasing marginal utility, is actually based on K. Marks, F. Engels, and J. Schumpeter's illustrations about the development miracle of the open market economy. Our research includes: (1) Combining their theories with the practice of enterprise's development under the new environment of new times, new economy conditions, and new productive forces; (2) Their theories about enterprise's activities under the conditions of the open market economy. The most important point of their theories is "innovation". A new microeconomics theory, the law of increasing marginal utility, can be concluded based on a series of K. Marks, F. Engels, and J. Schumpeter's theories. This new theory not only analyzes how Xinfei Corporation breaks through the law of diminishing marginal utility, but it also combines a new essential factor, "the knowledge", with productive forces, thereby forms the law of increasing marginal utility. The development miracle of Xinfei Corporation is the innovation machine described in the law of increasing marginal utility, and the inexhaustible power source for any company's development.

2.2 The application of the law of increasing marginal utility

The law of increasing marginal utility based on the research of Xinfei Corporation has following practical applications:

(1) Design "market innovation machine" under the knowledge economy system. Make corporations develop based on this law. Create a new miracle of microeconomics growth.

(2) Show the most important difference between the knowledge economic system and the other economic systems, i.e. forcing companies to unceasingly innovate by the pressure of market economy. The effect released by innovation is to break the traditional economic law of diminishing marginal utility, to prove the new economic law of increasing marginal utility, to create much more social wealth, and to make society progress rapidly.

(3) Construct a theory system based on the law of increasing marginal utility. In the future, under the knowledge economy, the competition between companies will no longer focus on the price, but on the innovation. The mode, "compete → innovate → re-compete", results in a miraculous economic development. Therefore, in other words, the knowledge economy can be considered as a kind of cyclic economy. And the law of increasing marginal utility for the 21st century can also be concluded as a growing path: "innovate, innovate, and unceasingly innovate."

(4) Construct a new mode for the enterprise management. The purpose of researching Xinfei Corporation is to research its innovation and its management mode with combining hard technology and soft technology together. Based on the law of increasing marginal utility, a new business management mode,

"21st Century Three-Color Business Management", will be proposed.

3. The fundamental of the conjecture: five research subjects and ten propositions

We have studied Xinfei's miraculous development, Xinfei's road of new industrialization, and Xinfei's innovative management mode and effect. But the most important point is to construct the theory system based on the law of the increasing marginal utility, and to prove its scientificalness and expansibility. Innovation is the core of this theory system.

3.1 Five research subjects

Subject 1. Research of the core of the modern innovation: The integration of the engineering technology and the modern business management technology.

Subject 2. Research of the mechanism of the modern innovation – the profit-driven mechanism: the value of technology and business trade.

Subject 3. Research of the business resource configuration of a company and the applicable mode of the innovation. It can prove the overflow effect of the innovation and the cyclic economy system. In other words, it is the research of the process of knowledge economy.

Subject 4. Research of the economic effect of "the innovation machine". This research can prove the relation between marginal utility and the increasing profit.

Subject 5. Research of the development of "the innovation machine". This research can explain the relation between the innovation and the business benefits. It can also be used in the process of constructing a harmonious society.

3.2 Ten propositions for the law of increasing marginal utility

We firstly research the knowledge economic system itself and the difference between this system and other economic systems, then explain the development miracle of enterprise by innovation, and solve the competition, the innovative change, the production innovation, the innovative spirit of entrepreneurs, the system of innovation, the creation of harmonious society, and the technological innovation based business exchange. All of these problems can be solved by the theory system of the law of increasing marginal utility based on following ten propositions.

(1) The knowledge economy is the basic economy obtained by enterprise, having amazing increasing rate, and having the characteristic of the law of the marginal utility. It is so-called the cyclic economy. The cyclic economy includes four circulations: a) macroeconomics circulation, develop → innovate → re-develop; b) microeconomics circulation, develop → invest → re-develop; c) integrated circulation for production elements, integrate → select → re-integrate; d) combinational circulation for technology and production, product → research → re-product.

(2) "The innovation machine" is the core of the microeconomics theory, including five parts: a) the value of the innovation; b) the competition of the innovation; c) the mechanism of the innovation and its revolution; d) the circulation and the cyclic economy based on the innovation; e) the relation between the innovative motivation and the innovative profit.

(3) The "technological innovation" consistency for the personal profit and the social wealth: a) The overflow effect of the technological innovation; b) the relation between the technology communication and

the driven-based innovation; c) the exchange value of the technological innovation; d) the league and monopoly for the trade of the technological innovation; e) the new mode for the technological innovation and the investment.

(4) The technology trading market for enterprises: a) the inner technological monopoly for an enterprise; b) the market value of the technology trading; c) the inner and outer effects of the technology trading; d) the role of market and market pressure in the technology trading.

(5) The substitution relation between the innovative driven factor and the personal profit: a) the relation between the overflow effect of the innovation and the market pressure; b) the united innovative system consisting of the marginal utility, the total cost, and the social benefit; c) the analysis for the optimal model of the innovation.

(6) The compensation for the enterprises' competition and innovation: a) the status and function of the modern management technology; b) the new competition model for enterprises (the competition not for price; c) the environment and effect of the innovation for production and process; d) the effect of the successful innovation for the social welfare.

(7) The effect of the marginal utility for "the innovation machine": a) the mechanism for the compensation of the innovation's cost; b) the relation between the innovation and the price (pressure); c) the marginal cost and the maximum profit; d) the cost efficiency for the continuous large scale economy; e) the rotating balance and the enterprise's spiral development; f) the centralization of the innovation and the release of the market effect.

(8) The core of the elements of production is the knowledge capital: a) the knowledge itself is a kind of capital; b) the innovation of the knowledge capital and the energy effect of the financing innovation; c) the function of the mechanism of the knowledge economy, i.e. the integration of price, innovation and information.

(9) The optimal schedule model for enterprises. This is the second model of the law of increasing marginal utility, for how to choose the innovation and how to optimize the innovation: a) the structure of the optimal selection model; b) the effect of the optimal schedule; c) the optimal model for the combination of the optimal schedule model and the optimal relation.

(10) The theory for the rules and the regulations of enterprises, which can guarantee the rapid development of enterprises: a) the trade-off between the rule and the efficiency of the profit; b) the trade-off between the innovation and the communication; c) the resource configuration of the technological innovation trading; d) the efficiency of assignment for the innovation and the technological communication.

4. Expected research model and the business management pattern based on the innovation

The expected results are the law of increasing marginal utility and the business management pattern based on this law, which consist of following contents:

(1) the innovation of the law of increasing marginal utility;

(2) the state-of-the-art economic theory based on this law;

(3) the business management pattern based on the innovation, including humanism, technology and the innovation machine, which is so-called "Red-Blue-Green" Three-Color Business Management;

(4) the model of the law of increasing marginal utility.

4.1 The description and the model of the law of increasing marginal utility

This model is a theory for describing the economy increase, and belongs to microeconomics.

(1) The important research of this model is the increase of economy. The input variables of the model are technology, human resource, and capital. The output is the increment of economy. This model shows the function of economic activities and economic relations.

(2) This model is described by a differential equation. This differential equation shows the relation between input variables, time and some specific conditions. It illustrates the increasing property of the economy.

(3) Denote input variables C, M, N and K as control variable, dynamic human variable, technology variable, and capital respectively. *MaxV* means the optimal increasing economy [3].

(4) The mathematic model is as: $MaxV = \int_0^T u[M(t) \cdot N(t) \cdot K(t) \cdot C(t) \cdot T]dt$, where *t* is time, and *u* is the basic function [4].

4.2 "Red-Blue-Green" Three-Color Business Management

4.2.1 Red Business Management

The red business management considers technology as the management core, converts economic resources from one place with lower productivity to another place with higher productivity, and realizes the theory of "Technology is the first productivity". The economy of the USA in 1990s can be considered as an economy for startup. Knowledge, technology and creativity are its three power sources. The purpose of business management is converting knowledge into wealth. Therefore, creation becomes the main behavioral path of technology. In other words, the "creation" is the process of the activity of red business management. The environment of red business management consists of six risks: risk of chance, risk of technology, risk of market, risk of capital, risk of management, and risk of environment. The creation can be divided into four stages: recognizing and estimating the technological chances in market; designing the creative management; creating and acquiring resources; creating business management. The four fundamental factors of red business management are: creator, human resource, technology, and capital.

4.2.2 Blue Business Management

Blue business management is a kind of management transforming the management of quality to the management of culture. Its main characteristics are:

(1) it is a human-based business management;

(2) it is a standard management for how people should think and behave in a specific society;

(3) it is a management combining ideology into development of material;

(4) it is a blue culture-based management;

(5) it is a management to show "the law of increasing marginal utility" in knowledge economy.

In one word, blue business management can release a huge effect of wealth. Its theoretical structure includes:

(1) the value of an organization is the core;

(2) the purpose is the huge profit increase;

(3) the main body of blue management system is intellectual resource;

(4) the method of blue management is to enlarge people's intelligence by innovative machines;

(5) the basic mode of blue business management is a kind of management based on humanity.

4.2.3 Green Business Management

Green business management is to guide an enterprise to develop under a reasonable environment. The main functions of green business management are realizing large-scale management, diversifying contents of management, and guaranteeing an enterprise to safely develop. Its theoretical structure including:

(1) creating a good environment for development;

(2) optimizing market resources for management;

(3) combining technology, capital and traditional management theories into a new integrated pattern for the modern business management;

(4) this modern business management consists of ten innovation systems (thought innovation, rule innovation, stimulus innovation, dynamic innovation, production innovation, method innovation, strategy innovation, decision innovation, organization innovation, and technology innovation).

(5) the new pattern and systems are the product transformation system, technological innovation system, management innovation system, capital innovation system, human stimulus system.

5. Conclusion

The law of increasing marginal utility and its ten propositions are considered as economic "Goldbach Conjectures". They are an innovative theory of economics. Based on this theory, we can construct the movement of knowledge economy era, create a new Three-Color business management mode, and find a new path for enterprises to generate much more profit than ever before.

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Received: 9/13/2007

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The Progress of IFN and the Correlation with Hepatitis B virus

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Abstract: IFN affects a number of processes including those regulating cell growth, differentiation, and apoptosis, as well as the modulation of the immune response. This review focuses on the mechanism of IFN in treatment and the correlation with Hepatitis B virus. [Nature and Science. 2007;5(3):78-81]. (ISSN: 1545-0740).

Keywords: Interferon, Hepatitis B virus

Interferon

Interferon (IFN) was discovered as an antiviral agent during studies on virus interference [1,2]. Isaacs and Lindenmann first reported in 1957 that influenza virus-infected chick cells produced a secreted factor that mediated the transfer of a virus-resistant state active against both homologous and heterologous viruses [1]. This seminal observation, along with similar findings described by Nagano and Kojima in 1958 [2], set the stage for subsequent studies that led to the elucidation of the IFN system in exquisite detail[3].

The IFN system has three types of interferon. IFNs were approved as therapeutics and moved from the basic research laboratory to the clinic. Advances made while elucidating the IFN system contributed significantly in multiple areas of mammalian cell biology and biochemistry, ranging from pathways of signal transduction to the biochemical mechanisms of transcriptional and translational control to the molecular basis of viral pathogenesis.

The system includes cells that synthesize IFN in response to an external stimulus such as viral infection and cells that respond to IFN by establishing an antiviral state[4,5,6]. Animal viruses are inducers of IFN, and are also sensitive to the antiviral actions of IFNs. Some animal viruses encode products that antagonize the IFN antiviral response. The IFN response represents an early host defense, one that occurs prior to the onset of the immune response. IFNs possess a wide range of biological activities in addition to the characteristic antiviral activity by which they were discovered[7].

Success in the basic research laboratory led to the subsequent utilization of IFNs as therapeutics in the clinic. Three kinds of human IFNs, IFN-α, IFN-β, and IFN-λ, have been approved for clinical use by the Food and Drug Administration. Diseases of known viral origin for which IFN-αspecies are most widely used are hepatitis C and hepatitis B (Table 1)[3].

TABLE 1. IFNs approved by the Food and Drug Administration for the treatment of viral hepatitis

IFN	Trade name	Manufacturer	Disease
IFN-α2a	Roferon A	Hoffman LaRoche	Hepatitis C
IFN-α2b	Intron A	Schering	Hepatitis B, Hepatitis C
IFN-αnl (lympho-blastoid)	Wellferon	Glaxo Wellcome	Hepatitis C
IFN-αcon	Infergen	Amgen	Hepatitis C
Peg-IFN-α2b	PEG-Intron	Schering	Hepatitis C

Tremendous progress has been made in understanding the molecular basis of the antiviral actions of IFN, and the strategies that viruses have evolved to antagonize these actions. The actions of IFN are pleiotropic and affect many biological processes in addition to the multiplication of viruses.

Hepatitis B virus

Hepatitis B virus (HBV) is an enveloped virus associated with significant morbidity and mortality[8,9]. HBV causes both acute and chronic liver disease.

The genome of HBV is a partially double-stranded circular 3.2 kb DNA molecule. The DNA genome of the virus is transcribed in the nucleus of the hepatocyte to produce the 3.5, 2.4, 2.1 and 0.7 kb viral transcripts [9,10]. The level of these transcripts are regulated by the enhancer 2/core promoter, the large surface antigen promoter, the major surface antigen promoter and the enhancer 1/X gene promoter, respectively [11,12,13].The 3.5 kb pregenomic HBV transcript encodes the viral polymerase and the nucleocapsid or core polypeptide[14]. The HBV polymerase binds to the packaging sequence (ε) in the pregenomic RNA and this complex is encapsidated by core polypeptides to form an immature viral capsid[15,16,17]. The reverse transcriptase/DNA polymerase activity of the viral polymerase converts the pregenomic RNA into the partially double-stranded DNA genome present in the mature capsid[18]. The mature capsid interacts with the surface antigen polypeptides and subsequently buds into the lumen of the endoplasmic reticulum as a virus particle[19,20,21]. Virions pass through the endoplasmic reticulum and Golgi apparatus prior to release from hepatocytes[22].

Infection with hepatitis B virus is a significant public health concern. Worldwide, an estimated 2 billion people are infected with the hepatitis B virus (HBV)[23].1 A total of 350 million people have the chronic form of hepatitis B infection, 75% of whom live in Asia[24].

most acquired the disease by vertical transmission or during preschool childhood. In the absence of vaccination most exposed neonates and young children will be infected and become lifelong carriers (Fiureg 1)[25]. Chronic infection, particularly of males, is often complicated by the eventual development of cirrhosis and then liver failure or hepatoma. In contrast, primary exposure of adults to hepatitis B virus typically causes acute resolving infection with clearance of the virus (Figure 1).

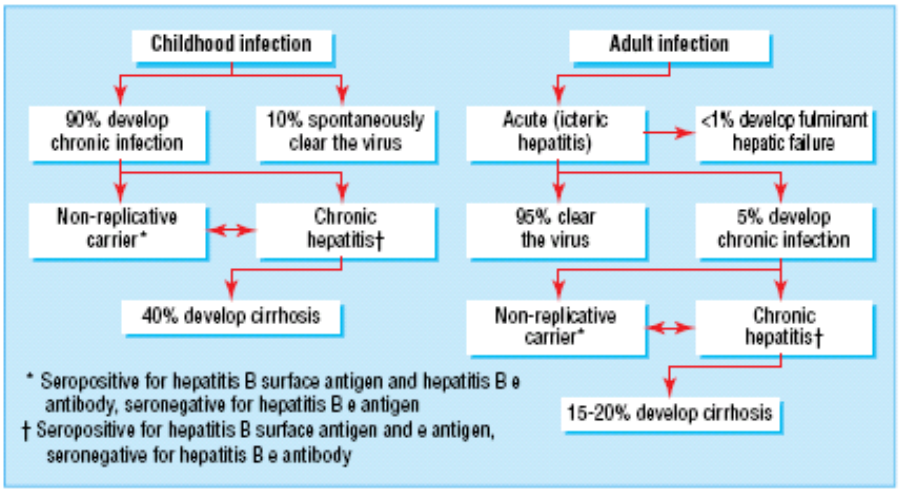

Fig 1 Natural course of primary hepatitis B infection acquired during childhood or adulthood

Chronic infection increases the risk for primary liver cancer. Endemic hepatitis B infection in Asia's large population contributes to primary liver cancer's position as the fourth leading cause of cancer death worldwide [26,27,28].

Interferon treatment

Interferon treatment (5 million units three times weekly for four to six months) increases the conversion rate from high level to low level viral replication in up to 15-20% of patients a year. Response rates are enhanced by higher doses, but the safety and tolerability of high dose interferon are a concern.

Chronic HBV infection is associated with liver cirrhosis and hepatocellular carcinoma[10,11]. A widely used therapeutic intervention is treatment with interferon α (IFN-α). IFN-α therapy can reduce viral replication and liver damage associated with viral infection.

Until recently, interferon alfa (IFN-α)was the only drug licensed for the treatment of chronic hepatitis

B. Interferon treatment is intended to inhibit viral replication and to augment the clearance of virally infected hepatocytes. Among patients with chronic infection, about 5% a year undergo spontaneous conversion from a state of high level viral replication to low level replication.

IFN-α is also generally recognized as the most important therapeutic agent in chronic hepatitis C[29]. In fact, normalization of alanine aminotransferase (ALT) levels and improvement in chronic liver inflammation and necrosis are reported in approximately 50-60% of patients. However, 50% of these responder patients are known to relapse at the end of therapy [30,31].

Conclusion

The use of IFN system reagents in studies of the virus host interaction, both in cell culture and in intact animals, continues to provide seminal contributions not only of mechanisms of viral pathogenesis but also of mechanisms of signal transduction and the transcriptional and translational control of macromolecular synthesis that is so important in many areas of mammalian cell biology and virology. The emergence of new tools and approaches for study of the virus-host interaction, including functional genomics and proteomics, together with advances in the areas of structural biology and combinatorial chemistry for the identification of novel molecules that affect the function of viral and cellular targets, should lead to further insights into the structure-function relationships for the IFN system components.

Received: 9/17/2007

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Progress in the Research of Insulin-like Growth Factors and the Binding Proteins

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Abstract: Insulin-like growth factors are the very important growth factors. They interact with the receptors and the binding proteins. Insulin-like growth factors play very important regulated roles in the cell proliferation, growth and pathogenesis of cancers. In this paper we summarized progress in the research of Insulin-like growth factors and the binding proteins. [Nature and Science. 2007;5(3):82-86]. (ISSN: 1545-0740).

Keywords: Insulin-like growth factors; mitogenic; binding proteins

1. Introduction

In 1957, Salmon and Daughaday[1] first found that IGF-1 and IGF-2 could promote the cartilage to absorb 35s in sulphate. They named them as sulphation factors. In 1963, Froesch[2] described them as NSILA1 and 2.

In 1972, they were named as Somatomedin [3] in 1976, Rinderknecht and Humbel[4] isolated two active factors. They shared high degree of structural homology with insulin. They renamed them as insulin-like growth factor-1 and insulin-like growth factor-2 (IGF-1 and IGF-2). In 1978, Rinderknecht and Humbel identified the structure and characteristic of IGF-1 and IGF-2[5].

The IGF system is a complex network, consisting of the two IGF peptides (IGF-1 and IGF-2), two IGF receptors, eight well characterized IGF binding proteins (IGFBPs). In this article, we summarized the progress in the research of Insulin-like growth factors system..

2. Insulin-like growth factors family

Insulin-like growth factors (IGFs) consist of IGF-1 and IGF-2. Because they have high degree homology to insulin, they were called insulin-like growth factors. IGF-1 has 49% homology to insulin. IGF-2 has 47% homology to insulin. IGF-1 has 62% homology to IGF-2 in human. The IGF system is extremely complex.

IGFs have two forms[6] in serum in vivo: the protein complex, IGFs combine with IGF binding proteins, and the free form. IGFs can synthesize in different period and different tissues. The mainly resource is in liver, about 90% of total IGFs. Insulin-like growth factors play very important regulating roles in the cell proliferation, growth and pathogenesis of cancers with autocrine and paracrine two manners. They can promote cells from cell cycle G1 to S[7], and involve in the proliferation of cancer cells[8].

IGF-I and IGF-II play essential roles in cell metabolism, proliferation and differentiation and to this extent have major effects on fetal and postnatal development and organogenesis in mammals [9,10].

IGFs have the same function as insulin: cellular hypertrophy. But the consequences are different. The IGFs enhance the cell hypertrophy is requisite for cell survival, hyperplasia, and differentiation, and insulin enhances cell hypertrophy primarily as a means to increase nutrient stores[11]. They have distinct roles in regulating nutrient utilization. They have different receptor locations. These hormones can differentially regulate metabolism.

The activity IGFs is controlled by several inputs, such as energy intake, protein intake, body temperature, environment temperature, environment stress micronutrient[11] etc. These inputs not only regulate IGF secretion but also localization and circulating half-life by regulating IGFBPs levels.

2.1 IGF-1 and its biological function

Insulin-like growth factor-1 (IGF-1) is a single-chain polypeptide with 70 amino acid. The weigh of it is 7649Da[12]. Human IGF-1 locates on chromosome 12,and it has five extrons. IGF-1 has four domains,

they are domain B,C,A and D. It has highly conservation in mammal animals. IGF-I is growth hormone dependent, produced by the liver and extra hepatic tissues[13] . It stimulates DNA synthesis as a progression factor in the cell cycle[14]. IGF-1 plays an essential role in growth, differentiation, regeneration, and metabolism in all vertebrates[15].

In vivo, IGF-1 mimics the effects of growth hormone. IGF-1 is considered to be the major somatomedin in humans. In cells, IGF-1 mediates either short-term, insulin-like effects, which include metabolic effects such as stimulation of glucose uptake, glycogen, and lipid synthesis, or long-term mitogenic effects such as stimulation of protein, RNA, and DNA synthesis[16]. IGF-1 promotes mesoderm, adipocyte, neuron, oligodendrocyte, ovary, and testicular cell differentiation.

2.2 IGF-2 and its biological function

Insulin-like growth factor-2 (IGF-2) is a protein with 67 amino acid. The weigh of it is 7471Da. Human IGF-1 locates on chromosome11 and close to insulin. It has nine extrons and four promoters (Figure 1). IGF-2 also has four domains, they are domain B,C,A and D.

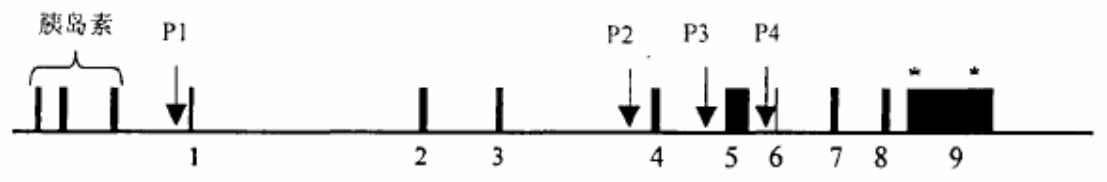

Figure 1. Insulin-like growth factor-1 (IGF-1)

The main function of IGF-2 in humans is not clear. In rodents, IGF-2 may function as a fetal growth factor; the levels of IGF-2 in fetal rat plasma are high and decline after birth[17]. IGF-2 values in adults are about four times higher than those of IGF-I[18].

The secretion of IGF-2 is usually high in the fetus. The concentration of IGF-2 will decline to different degrees after birth. It is synthesized primarily by the liver, but it is also produced locally by many tissues, where it acts an autocrine or paracrine manner.

IGF-2 stimulates glycogenesis in 18-day-old fetal hepatocytes cultured in the presence of glucocorticoids and this stimulation is regulated by secreted IGFBPs, especially IGFBP-1, which is the predominant IGFBP secreted by these cells[19]

Recently, the researches on IGF-2 are related to its imprinted gene. Genomic imprinting is a method of gene regulation whereby a gene is expressed in a parent-of-origin dependent fashion[20]. Paternally expressed IGF-2 encodes for a critical fetal mitogen, and mice deficient in this growth factor have a dwarf phenotype

IGF-2 and H19 (Figure 2) are closely linked imprinted genes lying at the centromeric end of a 1Mb imprinted domain on mouse chromosome7[21]. They are expressed only from the paternal and the maternal allele, respectively.

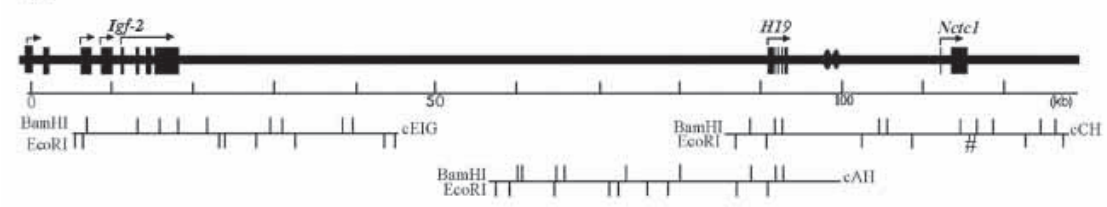

Figure 2. Insulin-like growth factor-2 (IGF-2)

3. Insulin-like growth factor binding proteins

In biological fluids, IGFs are normally bound to specific binding proteins, insulin –like growth factor binding proteins, IGFBPs[22]. They are belong to a structurally related secreted proteins family which has 8 members. IGFBPs specifically bind IGFs and modulate IGF bioactivity in different tissue[23]. They have two mechanisms: inhibitory mechanism and enhancing mechanism.

The IGFBPs deliver the IGFs to the cell surface, endowing the capability to IGF activity. IGFBPs and IGFs adhere to cell surfaces, their affinity drops and IGFs are released. The concentration of free IGFs

increased on the cell surface. It increases the potential for the cativation of IGF receptors and then expedites IGF activity.

Recently, multivalent cations were found [24] to control the adherence of IGFBP to cell surfaces. Both Zn2+ and La3+ (but not Mn2+) retain specific IGFBP on cell surfaces: IGFBP-3 on human GM-10 fibroblasts, IGFBP-3 on bovine MDBK kidney epithelial cells [24]. Zn2+ is also an important cation that controls IGFs access to cell. Some researches showed that the growth rate would decrease during Zn deficiency[25].

IGFBP-1, 25 to 34 kDa, is growth hormone-independent. It is the most important binding protein in amniotic fluid. Hepatic IGFBP-1 expression increases with stage of development, with a peak around birth[26], and is strongly enhanced in the presence of glucocorticoids[27]. IGFBP-1 is expressed largely in the liver and endometrium, suggesting that it plays some specific role in each organ [28.29].

IGFBP-2 , 32 to 34 kDa, is present in human cerebrospinal fluid, seminal plasma and lymph, in rat amniotic and cerebrospinal fluid.

The ability of IGFBP-1 and IGFBP-2 to expedite IGF activity requires a serum-derived factor [30]. The ability to expedite activity was directly related to the ability of these IGFBP to adhere to cell surfaces[30.31] . IGF-binding protein-2 was the predominant binding protein secreted by neonatal rat vascular smooth muscle cells. The predominant expression of IGFBP-2 in vascular smooth muscle cells from neonatal rats suggests that this protein may play a role in the development or growth of the vasculature and is consistent with the observation that IGFBP-2 is a major binding protein in fetal serum and fetal brain [32]. Generalized overexpression of IGFBP-2 in transgenic mice results in a 10–13% reduction of total body weight in adult animals[33].

IGFBP-3, 53kDa，is the most important binding protein. binds to IGF-1 or -2 with high affinity, can function as an inhibitor or activator of IGF-1 stimulated DNA synthesis. IGFBP-3 is a dominant binding protein in the blood in 40 times higher concentration than IGFBP-1 and with higher affinity to IGF-1. The majority of circulatory IGF-1 is bound to IGFBP-3[34]. Pioneer work by Harel and co-workers suggested that IGFBP-3 was also capable of inhibiting cell growth independently of its binding to IGFs [35].

IGF-binding protein-4 was most prevalent in adult vascular smooth muscle cells coincident with increased IGF-binding protein-4 protease activity[36].

IGFBP-5, 23 kDa, was purified by Andress and Birnbaum[37] from human osteoblast-derived culture. It stimulates osteoblast mitogenesis.

IGFBP-6, 30 to 32 kDa, was isolated from the human cerebrospinal fluid. It has high affinity to IGF-2 to IGF-1 about 10 times.

IGFBP-7,-8 have no affinity to IGFs, but have high affinity to insulin.

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Received: 9/17/2007

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Study of Gene Transfection Using Laser Irradiation and Increased Temperature Techniques

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Abstract: Gene therapy can be defined as the deliberate transfer of DNA for therapeutic purposes. The scientific objective of this study is to improve the gene transfer by laser method: (1) To develop the gene transfection system using laser beams. (2) To test the energy-transformation relationship between laser energy and gene transfer. (3) To make test the time-transformation relationship between laser time accumulation and gene transfer. (4) To get influence of laser on the cells after gene transfer. (5) To transfer genes into cells with laser method. (6) To transfer genes into worms in vivo. (7) To transfer genes into animal bodies in vivo. (8) Clinical trial to the gene therapy. [Nature and Science. 2007;5(3):87-90]. (ISSN: 1545-0740).

Keywords: cell; gene therapy; gene transfection; laser

1. Introduction

Gene therapy has reached a crossroads during the past years (Matsui, 2003). Gene therapy can be defined as the deliberate transfer of DNA for therapeutic purposes. There is a further implication in that it involves only specific sequences containing relevant genetic information. Transplantation procedures involving bone marrow, kidney and liver are not considered a form of gene therapy. The concept of transfer of genetic information as a practical clinical tool arose from the gene cloning technology developed during the 1970s (Bechtel, 1979). Without the ability to isolate and replicate defined genetic sequences it would be impossible to produce purified material for clinical use. The drive for the practical application of this technology came from the biotechnology industry, with its quest for complex human biomolecules produced by recombinant techniques in bacterial. Within a decade, pharmaceutical-grade insulin, interferon, interleukin-2 (IL-2) and tumor necrosis factor (TNF) were all undergoing clinical trials. The next step was to obtain gene expression *in vivo*. Genetic disorders were the obvious first target for such therapies. Abortive attempts were made in the early 1980s to treat two patients with thalassaemia (Temple, 1982). These experiments were surrounded by controversy as the pre-clinical evidence of effectiveness was not adequate and full ethical approval had not been given. For the features of a suitable target disease for gene therapy approaches, certain factors should be considered. The disease must be life-threatening so that the potential risk of serous side-effects is ethically acceptable. The gene must be must be available and its delivery to the relevant tissue feasible. This may involve the ex vivo transfection or transduction of cells removed from a patient, which are returned after maniputation. This approach is only possible with a limited range of tissues and most trials so far have used bone marrow. Ideally, a short-tern surrogate end-point to demonstrate the physiological benefit of the newly inserted gene should be available. The electrical conductance change in the nasal epithelium after insertion of the cystic fibrosis trans-membrane regulator gene is a good example. Finally, there must be some possibility that the disability caused by a disease is reversible. Some of the tragic mental and physical handicaps caused by some genetic metabolic disorders may never be improved by somatic gene therapy, however successful a gene transfer protocol. Gene transfer is one of the key factors in gene therapy. In this project, we will use gene gun as the tool to transfer human insulin gene into schistosoma, and consider calcium phosphate coprecipitation, laser gene transfer, etc. as the potential candidates.

Much interest has been shown in the use of lasers for nonviral targeted gene transfer, since the spatial characteristics of laser light are quite well defined (Ogura, 2004).

Shirahata et al made a small hole in a cell membrane by pulse laser irradiation to help a gene contained in a medium to be transferred into the cytoplasm through the hole. This hole disappears immediately with the application of laser irradiation of the appropriate power (Shirahata, 2001).

2. Research Design and Methods

Several techniques are currently used to transfer genes into various cells, tissues and organs. Although gene therapy is a potential therapeutic approach for arterial restenosis and angiogenesis, the efficiency of transfection is low regardless of the technique used. To transfer gene efficiently, a novel method laser radiation on gene transfection will be studied in this project. Pulse-wave Nd:YAG laser, Ho:YAG laser, UV excimer laser will be used.

(1) Cell culture: Mouse heart smooth muscle cells will be primarily cultured with a standard technique.
(2) Schistosome: Schistosome will be raised for gene transfection.
(3) Mouse: 100 mice will be housed for the gene transfection.
(4) Gene used: Gene with fluerescence label will be used in the project.
(5) Gene transfection: Gene transfection will be done using laser beam.

As other potential candidate, calcium phosphate coprecipitation method will be considered (Sambrook, 1989; Frederick, 1992; Ausubel, 1992)**:**

(1) **Growth of E. coli:**

Dissolve E. coli in 0.3 ml LB plus tetracycline (2 mg/ml) medium, transfer it into a tube containing 5 ml LB plus tetracycline (2 mg/ml) medium, 37^{o}C overnight, then freeze the E. coli (amplify in several tubes before freeze to get more samples).

(2) **Harvesting E. coli:**

A. Streak an inoculum across one side of a plate using sterile technique. Resterilize an inoculating loop and streak a sample from the first streak across a fresh part of the plate, then incubate at 37^{o}C until colonies appear (overnight).
B. Transfer a single bacterial colony into 2 ml of LB medium containing tetracycline (2 mg/ml) in a loosely capped 15-ml tube. 37^{o}C overnight with vigorous shaking.
C. Pour 1.5 ml of the culture into a microfuge tube. Centrifuge at 12,000g for 30 seconds at 4^{o}C in a microfuge. Store the remainder of the culture at 4^{o}C.
D. Remove the medium by aspiration.

(3) Lysis of E. coli:

A. Resuspend E. coli pellet in 100 μl of ice-cold Solution I (50 mM glucose, 25 mM Tris-Cl, pH 8.0, 10 mM EDTA, pH 8.0)
B. Add 200 μl of freshly prepared Solution II (0.2 N NaOH, 1% SDS), inverting the tube rapidly 5 times. Do not vortex. Store at 4^{o}C.
C. Add 150 μl ice-cold Solution III (5 M potassium acetate 60 ml, glacial acetic acid 11.5 ml, H_2O 28.5 ml), gently vortex, store on ice for 3-5 min.
D. Centrifuge at 12,000g for 5 min, 4^{o}C. Transfer the supernatant to a fresh tube.
E. Add 2 volumes of ethanol, Mix by vortex, keep at room temperature for 2 min.
F. Centrifuge at 12,000g for 5 min at 4^{o}C.
G. Remove supernatant and any drops of fluid adhering to the walls of the tube.
H. Rinse the pellet of DNA with 1 ml of 70% ethanol at 4^{o}C, then remove supernatant and any drops of fluid adhering to the walls of the tube.
I. Redissolve the DNA in 50 μl of TE (pH 8.0) containing DNAase-free pancreatic RNAase (20 μg/ml). Vortex briefly. Store at 20^{o}C.

(4) Purification of plasmid:

A. Transfer the DNA solution to a 15-ml Corex tube, and add 3 ml of an ice-cold solution of 5 M LiCl. Mix well, and then centrifuge at 10,000 rpm for 10 min at 4^{o}C.
B. Transfer the supernatant to a fresh 30-ml Corex tube. Add an equal volume of isopropanol. Mix well. Recover the precipitated DNA by centrifugation at 10,000 rpm for 10 min at room temperature.

C. Decant supernatant carefully, and invert the open tube to allow the last drops of supernatant to drain away. Rinse the pellet and the walls of the tube with 7% ethanol at room temperature. Drain off the ethanol entirely.
D. Dissolve the pellet in 500 μl of TE (pH 8.0) containing DNAase-free pancreatic RNAase (20 μg/ml). Transfer the solution to a microfuge and store at room temperature for 30 min.
E. Add 500 μl of 1.6 M NaCl containing 13% (w/v) polythylene glycol (PEG 800). Mix well. Recover the plasmid DNA by centrifugation at 12,000g for 5 min at 4°C.
F. Remove supernatant. Dissolve the pellet of plasmid DNA in 400 μl of TE (pH 8.0). Extract the solution once with phenol, once with phenol:chloroform, and once with chloroform.
G. Transfer the aqueous phase to a fresh microfuge tube. Add 100 μl of 10 M ammonium acetate, and mix well. Add 2 volumes (~1 ml) of ethanol, and store at room temperature for 10 min. Recover the precipitated plasmid DNA by centrifugation at 12,000g for 5 min at 4°C.
H. Remove the supernatant. Add 200 μl of 70% ethanol at 4°C. Vortex briefly, and then centrifuge at 12,000g for 2 min at 4°C.
I. Remove the supernatant, and store the open tube on the bench until the last visible traces of ethanol have been evaporated.
J. Dissolve the pellet in 500 μl of TE buffer (pH 8.0). Measure the $OD_{260\ nm}$ of a 1:100 dilution (in TE, pH 8.0) of the solution. Calculate the concentration of the plasmid DNA: 1 $OD_{260\ nm}$ = 50 μg of plasmid DNA/ml. Store the DNA in aliquots at -20°C.

(5) Transfer human insulin gene into schistosoma:
A. Gene gun method will be used in the gene transfer.
B. Detect: 12-48 hours after the addition of plasmid, measure the amount of human insulin product with ELISA. The primary antibody used in ELISA is specific active to swine hormone.

(6) Select the male worms with positive human insulin gene.

(7) Infect the male schistosoma transferred with schistosoma into human body.

3. Results

(1) Increased Temperature Enhanced Gene Transfer

The heated cultured human aorta smooth muscle cells had a significantly higher expression of the transfected swine growth hormone gene (Ma, 2004a). Incubated the swine growth hormone gene and human smooth muscle cells under the different incubation temperature of 23°C, 37°C and 43°C, the transfection increased with the temperature elevation ($p<0.01$). The greatest effects occurred within 10 min of incubation and persisted up to 30 min. In another experiment, we got the same result to transfer human interleukin-2 gene into cultured rat myocytes (Ma, 2004b). The results suggest that even a few degrees of ambient temperature rise can significantly increase gene transfer into cells. This may be of value when using gene therapy with transfection procedures.

(2) Laser Enhanced Gene Transfer

UV excimer laser ($XeCl_2$, 308 nm excimer laser, Spectranetics CVS-300™, Spectranetics, Colorado Springs, CO) was done with a 2.0 mm diameter optical fibers. Human aorta smooth muscle cells were cultured in F12K medium containing 2 mM glutamine, 10 mM HEPES, 10 mM TES, 50 ng/ml ascorbic acid, 10 μg/ml insulin, 10 μg/ml transferrin, 10 ng/ml sodium selenite and 30 μg/ml endothelial cell growth supplement, FBS 10% (Gibco BRL Life Technologies, Inc., Grand Island, NY, USA). Gene labeled with anti-amphiline mutation was used. The results showed that laser enhanced the gene transfer.

4. Discussions

Several techniques are currently used to transfer genes into culture cells and into various tissues and organs. These include electroporation, lipofection, calcium phosphate coprecipitation and DEAE-dextran, etc. Although gene therapy is a potential therapeutic approach for arterial restenosis following angioplasty, the efficiency of transfection is low regardless of the vector used. In our primary report it showed that when cultured human aorta smooth muscle cells and endothelial cells and perfused rat aorta artery in a chamber are heated at 45°C or 50°C for up to 1 or 2 hours, transient transfection by calcium

phosphate coprecipitation of a plasmid expressing human growth hormone is enhanced. Transfection of human interleukin-2 gene and swine growth hormone gene into the human smooth muscle cells and rat artery are also enhanced by heating. The results of our study suggest that the relatively low efficiency of gene transfer into tissues for therapy might be increased by short periods of heating during transfection (Ma, 2004a; Ma, 2004b). Also, the gene gun could be a effective technique to practice gene transfer.

5. Summary

The scientific objective of this study is to improve the gene transfer by laser method.

(1) To develop the gene transfection system using laser beams.
(2) To test the energy-transformation relationship between laser energy and gene transfer.
(3) To make test the time-transformation relationship between laser time accumulation and gene transfer.
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Volume 5 - Number 3 (Cumulated No. 16), September 10, 2007

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