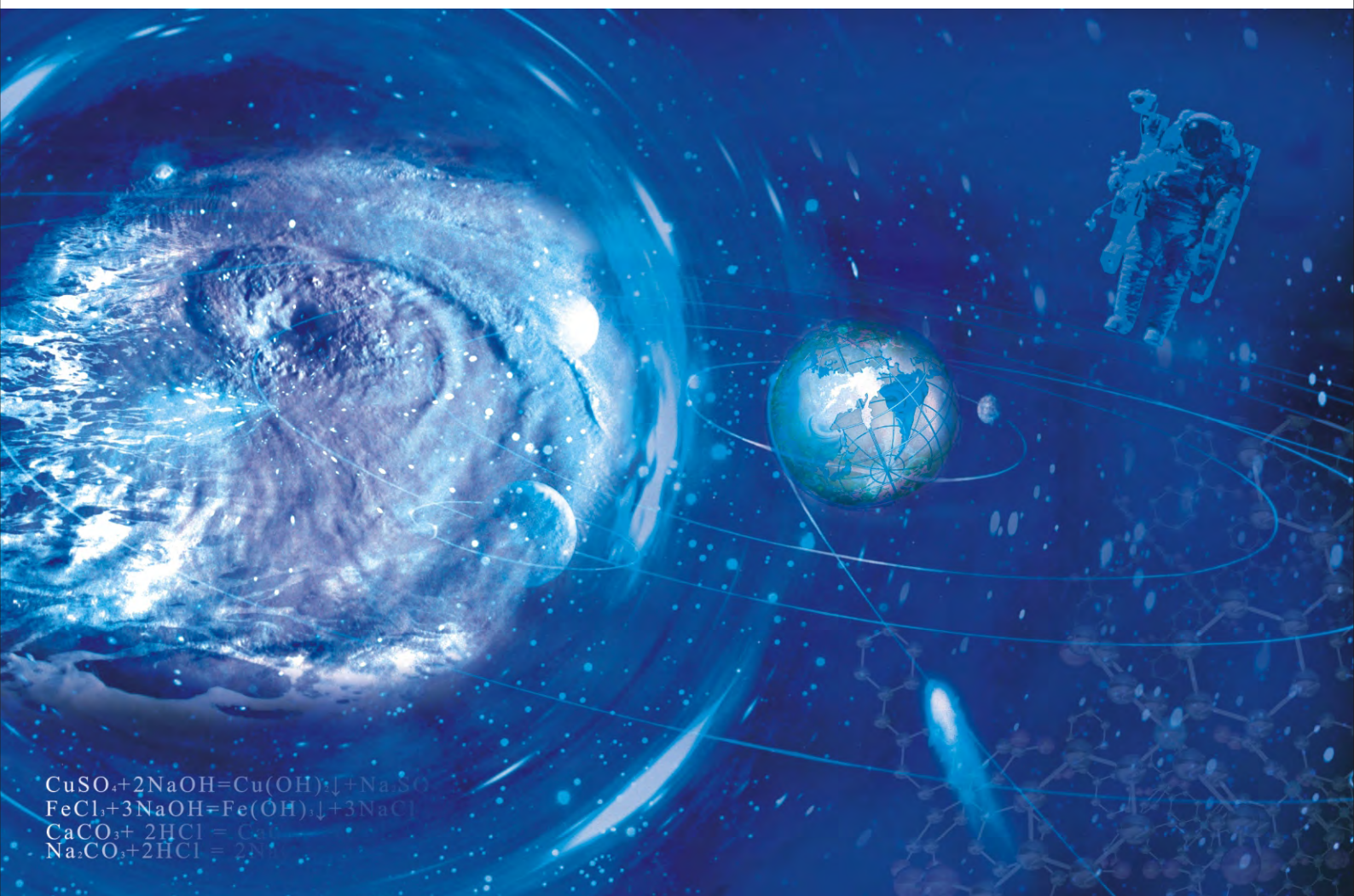




Natural Science



$\text{CuSO}_4 + 2\text{NaOH} = \text{Cu}(\text{OH})_2 \downarrow + \text{Na}_2\text{SO}_4$
 $\text{FeCl}_3 + 3\text{NaOH} = \text{Fe}(\text{OH})_3 \downarrow + 3\text{NaCl}$
 $\text{CaCO}_3 + 2\text{HCl} = \text{CaCl}_2 + \text{H}_2\text{O} + \text{CO}_2 \uparrow$
 $\text{Na}_2\text{CO}_3 + 2\text{HCl} = 2\text{NaCl} + \text{H}_2\text{O} + \text{CO}_2 \uparrow$

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News and Announcement

We are pleased to announce that one of the Editorial Board Members of **Natural Science**, Kuo-Chen Chen, has been identified by **Science Watch** (<http://sciencewatch.com/ana/fea/09maraprFea/>) as the author with the highest numbers of Hot Papers published over the preceding two years (2007 and 2008). Among the 13 authors listed in the table of “Scientists with Multiple Hot Papers” by **Science Watch**, Professor Dr. Kuo-Chen Chou of Gordon Life Science institute and Shanghai Jiaotong University ranks No.1 with 17 hot papers.

Meanwhile, the review article by Kuo-Chen Chou and Hong-Bin Shen of Shanghai Jiaotong University, entitled “Recent Progresses in Protein Subcellular Location Prediction” published in [Analytical Biochemistry](#), has been identified by **Science Watch** as the New Hot Paper in the field of Biology & Biochemistry (<http://sciencewatch.com/dr/nhp/2009/09marnhp/09marnhpChou/>). For more information about the hot research and hot papers, go to visit the web-sites at <http://www.sciencenet.cn/htmlnews/2009/3/216833.html>; <http://sciencewatch.com/ana/fea/pdf/09maraprFea.pdf>; and <http://sciencewatch.com/dr/nhp/2009/pdf/09marnhpChou.pdf>.

Please join us to send our warmest and sincere congratulations to our fellow board member, Kuo-Chen Chou, for his prominent contributions in science. Meanwhile, we hope this announcement can attract more researchers to submit their best papers to **Natural Science**, the journal that publishes the highest quality of research and review articles in all important aspects of natural sciences.

We would also like to take this opportunity to announce that, owing to the large number of manuscripts that we are receiving, **Natural Science** has increased the publication frequency from quarterly to monthly as of February 2010.

Natural Science Editorial Office

Disinfection of swimming pools with chlorine and derivatives: formation of organochlorinated and organobrominated compounds and exposure of pool personnel and swimmers

Maria-Cristina Aprea, Bruno Banchi, Liana Lunghini, Massimo Pagliantini, Antonio Peruzzi, Gianfranco Sciarra

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ABSTRACT

Chlorination of pool water leads to the formation of many by-products, chloroform usually being the most abundant. The paper reports the results of a study evaluating exposure of bathers and pool employees to trihalomethanes (chloroform, bromodichloromethane, dibromochloromethane, bromoform) in four indoor swimming pools with chlorinated water. Chloroform concentrations in environmental air samples when the pool was in use (about 9 h), in the range 1-182 $\mu\text{g}/\text{m}^3$, were greater near the pool than in the change rooms, passageways and offices. Chloroform concentrations in personal air samples of pool employees were in the range 18-138 $\mu\text{g}/\text{m}^3$. Urinary concentrations of chloroform averaged (geometric means) 0.123 and 0.165 $\mu\text{g}/\text{l}$ and 0.404 and 0.342 $\mu\text{g}/\text{l}$ prior and at the end of exposure during in water and out of water activities, respectively. The significant increase in urinary excretion of chloroform confirms that the source of the contaminant was pool water. Absorption of chloroform, estimated from airborne and water concentrations, was significantly correlated with delta chloroform (after/before exposure) and urinary concentrations of chloroform at the end of exposure. As chloroform is a toxic and possibly carcinogenic substance, these observations pose a problem principally for the general population of pool users.

Keywords: Disinfection By-Products; Indoor Swimming Pool; Trihalomethanes; Biological Monitoring; Exposure; Urine

1. INTRODUCTION

This paper is concerned with bathing complexes consisting of one or more artificial pools for recreational, educational, sporting or therapeutic activity carried out in the water. From the health point of view, pools are classified according to environmental and structural characteristics and type of use. Various documents defining guidelines for safe use of recreational facilities such as pools have been published [1-3]. Chemical hazards associated with frequentation of pools are summarized in **Figure 1**.

Chemical agents in pool water depend on the type of water used to fill the pool. Town water, for example, may contain organic matter and by-products of disinfection from previous treatments. Among the chemical agents derived from bathers, nitrogen compounds, especially ammonia, react with free disinfectants to form various by-products. Nitrogen compounds may come from skin secretions: the nitrogen content of sweat is about 1 g/l as ammonia, amino acids, creatinine and urea. Significant quantities of nitrogen compounds can come from urine: urine release by bathers averages 25-30 ml/person [4] but may exceed 77.5 ml/person [5]. No information is currently available about concentrations of compounds from cosmetics. With regard to chemical agents from maintenance, a considerable number of compounds are used to keep water quality acceptable. Disinfectants are added in order to deactivate pathogenic microorganisms. Chlorine in one of its various forms is the most common. Other disinfectants such as ozone and UV radiation kill or inactivate microorganisms at the time of treatment but do not have any residual effect that continues to act in the water. They are therefore used with chlorine or bromine to provide continuous disinfection. Chlorine dioxide is not considered a chlorine disinfectant as it acts differently without producing residual chlorine, through conversion to chlorite

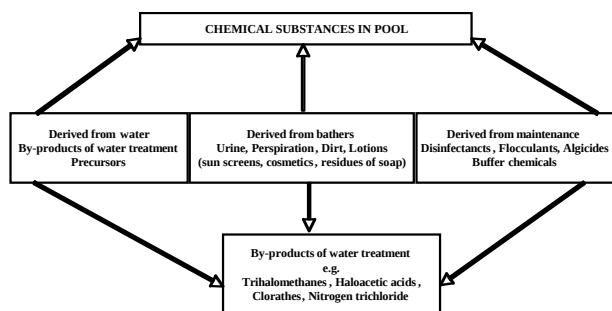


Figure 1. Summary of possible sources of chemical contamination in swimming pools.

and chlorate ions that remain in solution. Liquid bromine is seldom used, whereas sodium bromide and its oxidant (hypochlorite) are more common. Disinfection with bromine compounds is not suitable for outdoor pools because sunlight destroys bromine residues. In all cases, the choice is based on efficacy in the particular circumstances of use, as well as ease of handling and monitoring. Compounds used to correct pH depend on the type of disinfectant and its acidity/alkalinity. Alkaline disinfectants such as sodium hypochlorite only require addition of an acid, which is generally sodium hydrogensulphate, carbon dioxide or hydrochloric acid. Acid disinfectants such as chlorine require addition of an alkaline substance which is generally sodium carbonate solution. At correct doses with maintenance of pH between 7.2 and 8.0, disinfectants should not have adverse effects on health. Flocculants such as polyaluminium chloride can be used to facilitate removal of dissolved or suspended substances and colloids. They trap the substances in flocculate that can be removed by filtration.

The formation of by-products of disinfection is related to the reaction of disinfectants with other chemical substances in the water. The most abundant by-products are trihalomethanes, such as chloroform, the most abundant, together with haloacetic acids of which di- and trichloroacetic acids are the most abundant [6]. The presence of inorganic bromides in the water can induce formation of bromine after oxidation, which can participate in the formation of by-products such as brominated trihalomethanes. Use of ozone in the presence of bromides can lead to formation of bromates that can build up in the water if turnover is poor. Limited information is available on ozonation and its by-products. Ozone can react with organic substances to produce oxygenated compounds such as aldehydes and carboxylic acids. Chlorine and bromine react very quickly with ammonia forming chloramines and bromoamines. Little data is available on the impact of UV on disinfection by-products when used with other chemicals, but UV does not seem to form by-products and appears to significantly reduce chloramine levels.

Exposure of pool personnel and bathers may occur by

ingestion of water, inhalation of aerosol or vapours and cutaneous absorption. The quantity of water ingested by swimmers depends on various factors, including experience, gender, age and type of activity. Estimates show that water intake is higher in children (37 ml) than adults (16 ml), and in men (22 ml) than women (12 ml) [7]. Bathers inhale the air in contact with the water surface. The volume of air inhaled depends on the intensity of physical activity and exposure time. Exposure by inhalation regards substances in vapour form released by the water and aerosols created also by swimmer-induced splashing and stirring of the water. Concentrations at different levels in the air above the pool depends on factors such as ventilation, the size of the building and air circulation. Skin, including eyes and mucous membranes, is extensively exposed to chemical agents in pool water. The intensity of skin absorption depends on a series of factors including contact time, water temperature and concentration of toxic compounds.

Many by-products of disinfection have proven to be mutagenic, genotoxic, carcinogenic, fetotoxic, hepatotoxic, renotoxic, neurotoxic and dysmetabolic [8]. Chloroform and bromodichloromethane are classified by the International Agency for Research on Cancer (IARC) as possible carcinogens for humans (group 2B) [9], whereas the American Conference of Governmental Industrial Hygienists (ACGIH) considers chloroform to be carcinogenic for animals with unknown relevance to humans (Class A3) [10].

Concentrations of trihalomethanes in pool water [11-32] and in the air above the pool [11,14-16,18,26, 27,33,34] was examined in several studies. Some authors investigated also the absorption of trihalomethanes during time spent at the pool by measuring blood concentrations of chloroform or those in alveolar air [14-16,29, 35,36].

The aim of the present study was to assess exposure levels of swimmers and pool personnel to chlorinated and brominated organic compounds in public indoor pools. Airborne concentrations were determined in different parts of the pool premises, and when possible, by personal air sampling and determination of urinary excretion before and after exposure. Levels of the same compounds were also determined in water, as well as microclimatic and plant conditions. Absorption of chloroform estimated from airborne and water concentrations were compared with the increase in concentrations in urine during exposure.

2. MATERIALS AND METHODS

2.1. Microclimatic and Plant Conditions

Four public indoor pools were monitored. All used drinking water from the town water supply. On days of monitoring, one or more water samples were taken for

determination of brominated and chlorinated organic compounds (chloroform, bromodichloromethane, dibromochloromethane, bromoform).

Pool 1: pool volume 470 m³ plus compensation tank 20 m³, disinfectant calcium hypochlorite 65% and occasionally sodium dichloroisocyanate, air intake 18000 m³/h with aspiration of 15000 m³/h (turnover about 83%). The plant was monitored five times in 2006-2008. On sampling days, mean relative humidity was 51%-68%, mean air temperature 23.5-28.5°C. Mean air speed was 0.10-0.23 m/sec. Water temperature was 28.6-29°C, pH 7.3-7.8, nitrates 8.3-15 mg/l, isocyanic acid 40-75 mg/l, turbidity 0.2-0.3 mg/l, suspended solids 0.9 mg/l and residual free chlorine 0.29-1.12 mg/l. Maximum number of users per hour was 50-60 and total daily users 150-200.

Pool 2: pool volume 476.3 m³ plus compensation tank 24 m³, disinfectant sodium hypochlorite, air intake 30000 m³/h (turnover about 40%). The plant was monitored three times in 2007-2008. Mean relative humidity on sampling days was 70-75%, mean air temperature 23.2-24.5°C, mean air speed 0.06-0.07 m/sec, water temperature 28.4-28.6°C, pH 6.9-7.3, nitrates 0.7-1.1 mg/l, isocyanic acid <20 mg/l, turbidity 0.1-0.6 mg/l, suspended solids <1 mg/l and residual free chlorine 0.81-2.59 mg/l. Maximum number of users per hour 60-100, total daily users 220-240 persons.

Pool 3: pool volume 700 m³ plus compensation tanks 60 m³ disinfectant sodium dichloro-S-triazine-trione (Dichloro 63), air intake 40,000 m³/h without circulation. The plant was monitored twice in 2007-2008. On sampling days, mean relative humidity was 70-75%, mean air temperature 24.5-25.5°C, mean air speed 0.03-0.05 m/sec. Water temperature in adult pool 29-30°C, pH 6.9, nitrates 14 mg/l, isocyanic acid 20 mg/l, turbidity 0.3 mg/l, suspended solids < 1 mg/l, vinyl chloride 5-8 µg/l and residual free chlorine 0.8-1.5 mg/l. Total number of users per day 250-300.

Pool 4: pool volume 400 m³ plus compensation tank 24 m³, disinfectant sodium dichloro-S-triazine-trione and sodium hypochlorite, air input not available, turnover about 30%. The plant was monitored twice in 2007-2008. On sampling days, mean relative humidity was 83-84%, mean air temperature 24°C, mean air speed 0.05 m/sec, water temperature 29-30°C, pH 7.5, nitrates 11.6 mg/l, isocyanic acid 25 mg/l, turbidity 0.5 mg/l, suspended solids < 1 mg/l, vinyl chloride 0.05-0.12 µg/l and residual free chlorine 1.5-1.8 mg/l. Total number of users per day 200.

2.2. Study Population

In the four bathing complexes, six lifeguards and four instructors were monitored: the former worked at the poolside and the latter in the water. Thirty-one bathers underwent biological monitoring (15 swimmers at dif-

ferent levels of expertise, four competitive swimmers and 12 persons enrolled water gym sessions). All filled in a questionnaire about personal details, weight, height, smoking and drinking habits, occupation. This information was used in the statistical analysis of the results. Before enrolment in the study, all subjects gave their informed consent.

2.3. Personal and Environmental Air Sampling

Personal air sampling during the work-shift was performed for poolside personnel by means of radial diffusion air samplers for chloroform assay (Radiello®). In the measurements conducted in 2008, parallel active sampling with carbon vials was carried out at a flow rate of 100 ml/min to determine chloroform, bromodichloromethane, dibromochloromethane and bromoform. Double sampling was carried out to assay bromine compounds, for which the manufacturer of diffusion samplers does not provide equivalent rates.

Fixed (environmental) sampling was carried out about 1.5 m from the pavement at the edge of the pools (3 or 4 samples per bathing complex per day), in the changing rooms, offices and passages between the changing rooms and the pool. Sampling lasting 24 h was carried out with diffusion samplers (Radiello®) and others lasting about 9 h (when the pool was in use) were done using active carbon vials at an air flow of 100 ml/min. The same contaminants as for personal samples were assayed. In Pool 3, the 9-h sampling was divided into two periods of 4.5 h (morning and afternoon) in order to detect any changes in concentrations of the contaminants in the various areas. In this case the data was used as such and after calculation of the weighted mean concentration over the whole period the pool was open.

For the analytical determination, samples were added with carbon disulfide containing deuterated benzene as internal standard and left in contact with the solvent for 30 min. The extract was injected in the GC/MS apparatus (EI-SIM electronic impact, single ion monitoring). The analytical limit of detection (LOD) was 0.1 µg/sample.

2.4. Estimation of Absorption of Chloroform

Absorption of chloroform was estimated for staff and bathers, summing the fractions derived from direct ingestion of water, inhalation of aerosols and vapours, and transcutaneous absorption. For absorption by ingestion, the results of Evans *et al.* 2001 [7] were used. According to the latter, water intake averages 22 ml/h for men 12 ml/h for women. Knowing the concentration of chloroform in pool water, it was possible to calculate the quantity ingested, assuming 100% absorption. The fraction derived from ingestion was assumed to be zero for pool-side staff.

The fraction derived from inhalation was estimated on the basis of time spent in the water, lung ventilation and median concentration of airborne contaminant at the poolside, corrected by a factor of 1.8 because the measurements were made 1.5 m from the pavement instead of 20 cm from the water's surface [11]. A lung retention of 59% was assumed, as proposed by Kuo *et al.* [37]. Lung ventilation was assumed to be 15 l/min for males and 12 l/min for females for tasks involving little exertion (lifeguard) and 30 l/min for males and 25 l/min for females for activity in the water. For poolside personnel, the concentration found by personal air sampling was used.

The fraction derived from skin absorption was estimated on the basis of time spent in the water, chloroform concentration in pool water, body surface area estimated on the basis of weight and height using the Du Bois formula [38] and 80% contact of the skin with water. The permeation constant of skin was assumed to be 0.2 cm/h as proposed by Kuo *et al.* [37]. The fraction derived from skin absorption was assumed to be zero for poolside personnel.

2.5. Urine Sampling

Spot samples of urine were obtained from personnel and bathers before and after exposure. Chloroform, bromodichloromethane, dibromochloromethane and bromoform were determined in all samples. The determination was performed analyzing the head space in GC/MS EI-SIM using deuterated benzene as internal standard. The LOD was 0.050 µg/l.

The data, expressed in µg/l was used as such and as differences between concentrations before and after exposure.

2.6. Statistical Analysis

Statistical analysis was done using Stat View 5.0, Power PC Version (SAS Institute Inc.). Values below the analytical limit of detection (LOD) were analyzed as half the LOD when at least half the data was over the LOD. Values above LOD but not quantifiable (<LOQ) were analyzed as the mean of LOD and LOQ. Parametric tests were used (analysis of variance, regression analysis, Student's t test for paired and unpaired data) and the level of significance chosen was $\alpha = 0.05$.

3. RESULTS

Concentrations of trihalomethanes in pool water on sampling days are shown in **Table 1**. Concentrations of contaminants in the water depended on the type of disinfectants used, on any impurities in the water used to fill the pool and on water characteristics. Brominated compounds seemed to be associated more with use of sodium hypochlorite than with compounds such as calcium hypochlorite or Dichloro 63, irrespective of isocyanates. Chloroform was confirmed to be the most abundant trihalomethane, and was only equal in concentration to bromodichloromethane and dibromochloromethane in pool 2, disinfected with sodium hypochlorite alone.

Table 1. Concentrations of trihalomethanes (µg/l) in water of four public pools.

		Chloroform	BDCM	DBCM	Bromoform
Pool 1	N	5	3	3	3
	N < LOD	0	0	3	3
	Mean ± SD	85.54±35.73	1.87±0.23	-	-
	Median	83.20	2.00	-	-
	GM	75.47	1.86	-	-
	Min-Max	35.7-127.00	1.60-2.01	-	-
Pool 2	N	3	3	3	3
	N < LOD	0	0	0	0
	Mean ± SD	12.33±2.10	17.73±1.56	17.67±2.61	4.60±1.13
	Median	12.40	17.90	17.40	4.0
	GM	12.21	17.69	17.54	4.52
	Min-Max	10.2-14.4	16.1-19.2	15.2-20.4	3.90-5.90
Pool 3	N	6	6	6	6
	N < LOD	0	1	0	0
	Mean ± SD	33.23±9.72	1.48±0.61	0.63±0.09	0.04±0.004
	Median	38.40	1.70	0.61	0.040
	GM	31.83	1.25	0.62	0.042
	Min-Max	19.00-40.80	0.25-1.80	0.52-0.78	0.40-0.50
Pool 4	N	6	6	6	6
	N < LOD	0	0	0	0
	Mean ± SD	11.98±0.80	3.10±0.14	1.47±0.05	0.17±0.02
	Median	11.70	3.10	1.50	0.18
	GM	11.96	3.10	1.47	0.17
	Min-Max	11.10-13.10	2.90-3.30	1.40-1.50	0.13-0.19

BDCM= Bromodichloromethane. DBCM= Dibromochloromethane. GM = geometric mean.

Table 2. Descriptive statistics of concentrations of chloroform ($\mu\text{g}/\text{m}^3$) detected in environmental air samples in four public pools.

TOTAL DATA						
	Poolside		Change rooms and offices		Passageways	
	9 h (a)	24 h (b)	9 h (a)	24 h (b)	9 h (a)	24 h (b)
N	26	40	15	23	3	5
N < LOD	0	0	7	2	1	0
Mean $\pm$ SD	85 $\pm$ 50	52 $\pm$ 30	11 $\pm$ 12	8 $\pm$ 7	34 $\pm$ 29	25 $\pm$ 9
Median	65	46	4	5	50	20
GM	70	43	5	6	14	24
Min-Max	21-182	12-127	1-34	1-29	1-52	18-36
POOL 1						
	Poolside		Change rooms and offices		Passageways	
	9 h (a)	24 h (b)	9 h (a)	24 h (b)	9 h (a)	24 h (b)
N	9	15	3	5	3	5
N < LOD	0	0	1	0	1	0
Mean $\pm$ SD	124 $\pm$ 41	81 $\pm$ 24	14 $\pm$ 11	7 $\pm$ 3	34 $\pm$ 29	25 $\pm$ 9
Median	128	78	18	6	50	20
GM	118	77	7	6	14	24
Min-Max	66-182	39-127	1-22	3-10	1-52	18-36
POOL 2						
	Poolside		Change rooms and offices		Passageways	
	9 h (a)	24 h (b)	9 h (a)	24 h (b)	9 h (a)	24 h (b)
N	9	9	6	6	0	0
N < LOD	0	0	3	0	-	-
Mean $\pm$ SD	35 $\pm$ 15	25 $\pm$ 10	5 $\pm$ 6	5 $\pm$ 1	-	-
Median	32	23	2	5	-	-
GM	33	23	3	5	-	-
Min-Max	21-62	12-39	1-14	4-7	-	-
POOL 3						
	Poolside		Change rooms and offices		Passageways	
	9 h (a)	24 h (b)	9 h (a)	24 h (b)	9 h (a)	24 h (b)
N	4	8	3	6	0	0
N < LOD	0	0	0	0	-	-
Mean $\pm$ SD	132 $\pm$ 11	56 $\pm$ 14	29 $\pm$ 4	18 $\pm$ 8	-	-
Median	130	53	27	16	-	-
GM	131	55	29	17	-	-
Min-Max	120-147	39-76	26-34	11-29	-	-
POOL 4						
	Poolside		Change rooms and offices		Passageways	
	9 ore (a)	24 ore (b)	9 ore (a)	24 ore (b)	9 ore (a)	24 ore (b)
N	4	8	3	6	0	0
N < LOD	0	0	3	2	-	-
Mean $\pm$ SD	61 $\pm$ 4	26 $\pm$ 12	-	4 $\pm$ 4	-	-
Median	63	26	-	3	-	-
GM	61	24	-	2	-	-
Min-Max	55-64	14-40	-	1-10	-	-

(a) Sampling conducted for 9 h in the presence of bathers; (b) Sampling conducted for 24 h in the presence and absence of bathers.

Chloroform concentration detected in environmental samples are summarized in **Table 2**, where the poolside, change room, office and passageway data is presented separately. The table also shows 9-h and 24-h sampling data separately. The sampling site and bathing complex significantly affected chloroform concentrations when the pools were open and over 24 h.

For 9-h sampling, the model explained 76% of the variance, with bathing complex explaining 31% and sampling site 45%, whereas for 24-h sampling the corresponding percentages were 70%, 28% and 42%. Twenty-four-hour values were less than those measured when the pools were open, especially at the poolside, indicating that movement of the water increased airborne

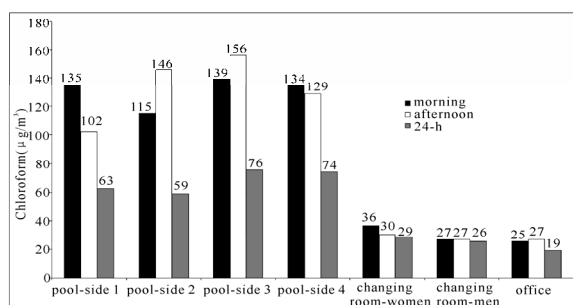


Figure 2. Concentrations of chloroform in morning, afternoon and 24-h environmental samples at pool 3.

chloroform.

Confirming this, **Figure 2** shows chloroform concentrations found in environmental samples at pool 3: sampling when the pool was open in the morning and afternoon gave values much higher than 24-h data at the poolside, whereas those obtained in changing rooms and offices did not vary.

Chloroform concentrations measured at the poolside when the pool was in use showed a statistically significant correlation ($p < 0.0001$) with chloroform concentrations in the water and with the number of pool users per day (N). Multiple regression analysis showed that 74% of the variance was explained according to the following Equation:

$$\text{CHCl}_3 \text{ air } (\mu\text{g}/\text{m}^3) = -101.6 + 1.70 \text{ CHCl}_3 \text{ water } (\mu\text{g}/\text{l}) + 0.57 \text{ N}$$

Temperature did not contribute significantly to the regression.

Chloroform concentrations in personal air samples in the three bathing complexes where they were obtained are summarized in **Table 3**. As expected, they were lower than at the poolside because these personnel did not spend the whole shift beside the pools but also spent time in offices etc. where airborne concentrations of the contaminant were often less.

With regard to airborne concentrations of brominated compounds, bromoform was never detected whereas bromodichloromethane (BDCM) and dibromochloromethane (DBCM) were only detectable in poolside samples of pools 2 and 4, where significant concentrations of the same contaminants were also found in the water (**Table 4**). The amount of data was therefore insufficient for multiple regression analysis between airborne and water concentrations. However, it can be said that the chemico-physical characteristics of contaminants strongly affect dispersal dynamics. In other words, for a given water concentration, chloroform passes much more readily into the vapour phase (vapour pressure 21.2 kPa at 20°C) than bromodichloromethane (vapour pressure 6.6 kPa at 20°C) and other brominated compounds that have even lower vapour pressures.

Concentrations of BDCM, DBCM and bromoform were undetectable in all urine samples. Descriptive statistics of chloroform concentrations detected in urine before and after exposure are summarized in **Table 5** which also shows differences in concentration (delta) between the two times. The data is separated for persons carrying on activity in the water, for whom inhalation, ingestion and cutaneous absorption are likely, and personnel working out of the water, for whom only respiratory exposure is likely.

A quick look at **Table 5** shows that delta after/before was higher for subjects carrying on activity in the water, confirming the hypothesis of skin and digestive absorption. In **Table 6**, the difference in concentrations after/before exposure is shown in a differentiated manner depending on the type of activity and/or the pool frequented. Despite the small number of data items available the table shows that for a given activity, delta after/before depended on the bathing complex and therefore on water and airborne concentrations of this contaminant.

Table 3. Descriptive statistics of concentrations of chloroform ($\mu\text{g}/\text{m}^3$) detected in personal air samples.

	TOTAL DATA	POOL 1	POOL 3	POOL 4
N	17	8	6	3
N<LOD	0	0	0	0
Mean $\pm$ SD	68 $\pm$ 35	89 $\pm$ 35	61 $\pm$ 17	25 $\pm$ 10
Median	61	95	61	20
GM	58	82	59	23
Min-Max	18-138	37-138	34-84	18-36

Table 4. Descriptive statistics of concentrations di bromodichloromethane (BDCM) and dibromochloromethane (DBCM) ($\mu\text{g}/\text{m}^3$) detected in poolside air samples of the two pools in which at least 50% of the data was detectable.

	POOL 2		POOL 4	
	BDCM	DBCM	BDCM	DBCM
N	9	9	4	4
N<LOD	2	3	0	4
Mean $\pm$ SD	16 $\pm$ 10	8 $\pm$ 6	10 $\pm$ 3	-
Median	18	8	9	-
GM	10	5	9	-
Min-Max	1-27	1-17	7-13	-

Table 5. Descriptive statistics of concentrations of chloroform ($\mu\text{g/l}$) detected in urine.

	Type of exposure (a)	Start of exposure (before)	End of exposure (after)	Delta after/before
N	In water	35	35	35
	Out of water	6	6	6
N<LOD	In water	11	0	-
	Out of water	1	0	-
Mean $\pm$ SD	In water	0.262 $\pm$ 0.351	0.659 $\pm$ 0.667	0.397 $\pm$ 0.455
	Out of water	0.265 $\pm$ 0.236	0.563 $\pm$ 0.627	0.297 $\pm$ 0.480
Median	In water	0.100	0.420	0.208
	Out of water	0.189	0.329	0.091
GM	In water	0.123	0.404	-
	Out of water	0.165	0.342	0.141
Min-Max	In water	0.025-1.676	0.025-3.327	0-2.227
	Out of water	0.025-0.615	0.100-1.746	0.052-1.271

(a) Exposure in water: swimmers, competitive swimmers, water gym participants, instructors; Exposure out of water: lifeguards and attendants.

Table 6. Differences between concentrations at the end and start of exposure ($\mu\text{g/l}$) detected in urine during activity in and out of the water by pool and specific activity.

	N	Mean $\pm$ SD	Median	GM	Min-Max
Competitive swimming (a)	4	0.065 $\pm$ 0.047	0.074	-	0-0.113
Total swimmers	15	0.506 $\pm$ 0.585	0.250	-	0-2.227
Pool 1	9	0.768 $\pm$ 0.635	0.672	0.490	0.025-2.227
Pool 4	6	0.112 $\pm$ 0.077	0.107	-	0-0.207
Water gym course	12	0.311 $\pm$ 0.326	0.204	-	0-1.301
Pool 3	8	0.231 $\pm$ 0.154	0.186	-	0-0.426
Pool 4	4	0.472 $\pm$ 0.554	0.223	0.309	0.142-1.301
Instructor	4	0.580 $\pm$ 0.225	0.511	0.550	0.408-0.887
Pool 1	3	0.637 $\pm$ 0.238	0.609	0.607	0.414-0.887
Pool 3	1	-	-	-	0.408
Lifeguard/attendant	3	0.529 $\pm$ 0.645	0.209	0.305	0.107-1.271
Pool 3	2	-	-	-	0.052-0.071
Pool 4	1	-	-	-	0.075

(a) only pool 3.

Table 7. Absorbed chloroform (μg) estimated on the basis of concentrations in water and in poolside air while the pool was open.

	N	Mean $\pm$ SD	Median	GM	Min-Max
Total doses	41	358.9 $\pm$ 301.9	173.6	267.2	109.4-1248.4
Doses ingested	35	1.4 $\pm$ 1.8	0.35	0.66	0.12-5.82
Transcutaneous doses	35	194.6 $\pm$ 217.5	85.5	108.4	25.4-844.0
Respiratory doses	41	191.5 $\pm$ 132.3	160.1	158.7	89.9-716.7
% ingested dose	35	0.3 $\pm$ 0.2	0.2	0.2	0.1-0.7
% cutaneous dose	35	42.5 $\pm$ 17.1	45.7	39.2	17.1-78.6
% respiratory dose	35	57.2 $\pm$ 17.2	54.1	54.3	17.2-77.9

Table 7 shows estimated absorbed doses of chloroform and the percentages of the total constituted by digestive, skin and inhalatory doses. Our estimates produced absorption values up to about 1.25 mg for swimmers or instructors in the water for long periods (2-3 h). For those carrying on activity in the water, the ingested percentage of the total dose was negligible compared to respiratory and skin doses that were 57% and 43%, respectively.

Estimates of absorbed dose were analysed by linear regression model with chloroform delta after/before exposure and urinary concentrations of chloroform at the

end of exposure. The results are shown in **Figures 3** and **4**. Both regressions were highly significant and the variance explained by the model was 53% and 71%. The intercept with the ordinate was very close to zero in **Figure 3**, as expected, and at 0.084 mg/l in **Figure 4**. In the latter case, the intercept should indicate the urinary concentration of chloroform not due to time spent in the pool (pre-exposure value) and indeed it was close to the median for urinary chloroform at the beginning of exposure for subjects carrying on activity in the water, shown in **Table 5** (this data was also the most numerous). The better correlation obtained in **Figure 4** between

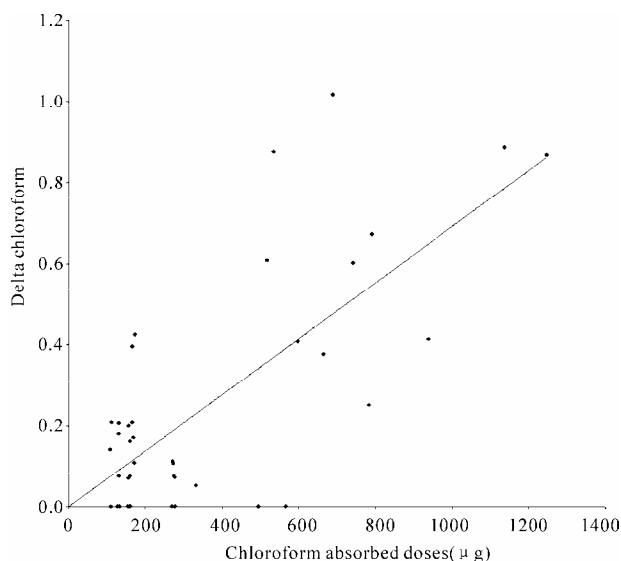


Figure 3. Linear regression analysis between estimated absorbed doses and delta chloroform after/before exposure ($y = 0.00069x + 0.0004$, $r^2 = 0.534$, significant $p < 0.0001$).

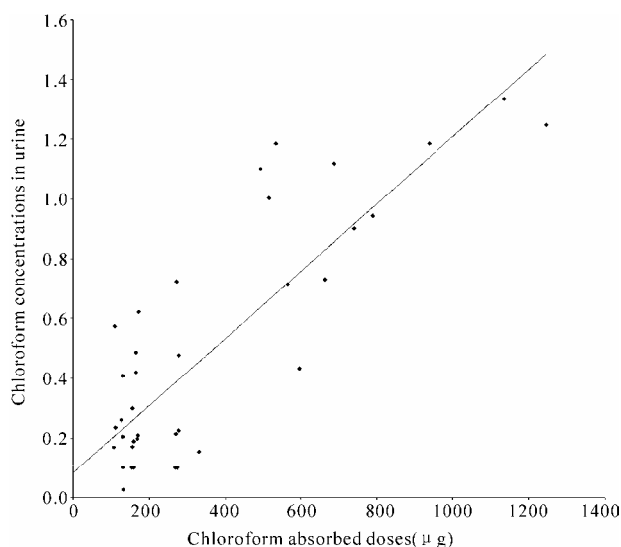


Figure 4. Linear regression analysis between estimated absorbed doses and chloroform concentrations in urine at the end of exposure ($y = 0.0011x + 0.084$, $r^2 = 0.706$, significant $p < 0.0001$).

estimated absorbed dose of chloroform and urinary excretion of chloroform at the end of exposure with respect to the delta for chloroform can probably be ascribed to the further factor of variability due in the second case to the pre-exposure value of chloroform. The slope of the two regressions was very low, probably due to the fact that chloroform eliminated in urine at the end of exposure is only a limited part of the total absorbed, whereas a greater fraction is presumably eliminated by exhalation and stored in body fat.

4. DISCUSSION

Concentrations of trihalomethanes reported in pool water vary from study to study but the results are not dissimilar to ours: Sandel [12] examined data from 114 home pools in the USA, obtaining a mean concentration of chloroform of 67.1 µg/l and a maximum of 313 µg/l. Most other available data on trihalomethanes in pool water is summarized in **Table 8**. In the pools monitored by us, formation of brominated trihalomethanes seemed prevalently associated with the use of sodium hypochlorite for water treatment. Ignoring bromide ions in the water used to fill these pools (town water in the case of pools 3 and 4), the presence of these compounds is presumably due to bromide impurities in the treatment reagents. This evidence makes it important to use only high purity reagents to treat town water.

Water-air transport of trihalomethanes depends on a number of factors that include concentrations in pool water, temperature and water disturbance and splashing by bathers. In our study, the concentration of chloroform detected at the poolside showed a good correlation with chloroform concentrations in pool water and with the number of swimmers present. Air and water temperatures were excluded from the regression model because they did not seem to have a significant effect on environmental concentrations of chloroform. Trihalomethane concentrations at different levels in the air above the pool should also depend on factors such as ventilation, size of pool building and air circulation. Most of the data available on concentrations of trihalomethanes in air above the pools is summarized in **Table 9**, which shows that measurements taken 20 cm above the water were on average 1.8 times higher than those taken 150 cm above the water.

In our study, concentrations of airborne trihalomethanes depended on where the measurements were taken (poolside, change rooms and offices, corridors) as found in other studies: Fantuzzi *et al.* [34] studied total trihalomethane concentrations in five Italian indoor pools, finding mean concentrations in poolside air of $58.0 \pm 22.1 \mu\text{g}/\text{m}^3$ and $26.1 \pm 24.3 \mu\text{g}/\text{m}^3$ at the reception.

Absorption of trihalomethanes during time spent at the pool was investigated by comparing urinary excretion of chloroform before and after exposure. Levels observed at the start of exposure were slightly less than detected in the general population. A study conducted in Italy in 1994 [39] found median concentrations of 194 ng/l in the rural population (115 subjects) and 490 ng/l in the urban population (87 subjects). Most previous studies on absorption of trihalomethanes at swimming pools measured blood concentrations of chloroform or those in alveolar air. Strähle *et al.* [35] compared concentrations

of trihalomethanes in blood of swimmers with those in pool water and air. The results, summarized in **Table 10**, demonstrate that inhalation is probably the main route of absorption of volatile components, since concentrations in water of indoor pools are greater than those of outdoor pools, while concentrations in ambient air are higher indoors, as are blood concentrations. Good ventilation of pool premises should therefore significantly reduce exposure. Erdinger *et al.* [29] confirmed that exposure is prevalently respiratory, showing a ratio of 3:1 with re-

spect to skin absorption. Aggazzotti *et al.* [14-16,36] showed that exposure in chlorinated pools can cause an increase in trihalomethane concentrations in plasma and alveolar air, but the latter declines soon after leaving the pool. Plasma concentrations of chloroform were detectable in 100% of the 127 samples analyzed, showing a mean concentration of 1.06 µg/l, whereas BDCM, detectable in only 25 samples, showed a mean of 0.14 µg/l and DBCM, detectable in only 17 samples, showed a mean of 0.1 µg/l.

Table 8. Concentrations of trihalomethanes in pool water (µg/l).

country	Chloroform		BDCM		DBCM		Bromoform		Type of pool	Ref.
	Mean	Range	Mean	Range	Mean	Range	Mean	Range		
Poland		35.9-99.7		2.3-14.7		0.2-0.8		0.2-203.2	Indoor	[13]
Italy		19-94								[14]
	93.7	9-179							Indoor	[15]
	33.7	25-43	2.3	1.8-2.8	0.8	0.5-10	0.1	0.1		[16]
USA	37.9								Indoor	[17]
		4-402		1-72		<0.1-8		<0.1-1	Outdoor	[18]
		3-580		1-90		0.3-30		<0.1-60	Indoor	
Germany	14.6	2.4-29.8							Indoor	[19]
	43	14.6-111							Outdoor	
	198	43-980	22.6	0.1-150	10.9	0.1-140	1.8	0.1-88	Indoor	[20]
		0.5-23.6		1.9-16.5		<0.1-3.4		<0.1-3.3	Indoor	[21]
		3.6-82.1		1.6-17.3		<0.1-15.1		<0.1-4.0	Outdoor	
	94.9	40.6-117.5	4.8	4.2-5.4	1.8	0.78-2.6			Indoor	[22]
	80.7		8.9		1.5		<0.1		Indoor	[23]
	74.9		11.0		3.0		0.23		Outdoor	[24]
		3-27.8							Indoor	[11]
		1.8-28							Indoor	[25]
Germany		8-11								[26,27]
	14	0.51-69	2.5	0.12-15	0.59	0.03-4.9	0.16	<0.03-8.1	Indoor	
	30	0.69-114	4.5	0.27-25	1.1	0.04-8.8	0.28	<0.03-3.4	Outdoor	
Denmark	3.8	6.4 max							Indoor	[28]
		7.1-24.8							Indoor	[29]
		145-151							Indoor	[30]
Hungary	11.4	<2-62.3	2.9	<1-11.4					Indoor	[31]
UK	121.1	45-212	8.3	2.5-23	2.7	0.67-7	0.9	0.67-2	Indoor	[32]

Table 9. Concentrations of trihalomethanes in air above the pool surface (µg/m³).

country	Chloroform		BDCM		DBCM		Bromoform		Type of pool	Ref.
	Mean	Range	Mean	Range	Mean	Range	Mean	Range		
Italy	214	66-650	19.5	5-100	6.6	0.1-14	0.2			[15]
	140	49-280	17.4	2-58	13.3	4-30	0.2		Indoor (a)	
	169	35-195	20	16-24	11.4	9-14	0.2			
Canada		597-1630							Indoor	[33]
	65		9.2						Indoor (a)	
	36		5.6		3.8				Indoor (b)	
Germany	5.6		0.21		1.2				Outdoor (a)	[11]
	2.3								Outdoor (a)	
	3.3	0.33-9.7	0.4	0.08-2.0	0.1	0.02-0.5	<0.03		Outdoor (a)	
	1.2	0.36-2.2	0.1	0.03-0.16	0.05	0.03-0.08	<0.03		Outdoor (b)	
	39	5.6-206	4.9	0.85-16	0.9	0.05-3.2	0.1	<0.03-3.0	Indoor (a)	
	30	1.7-136	4.1	0.23-13	0.8	0.05-2.9	0.08	<0.03-0.7	Indoor (a)	
USA		<0.1-1		<0.1		<0.1		<0.1	Indoor (c)	[18]
		<0.1-260		<0.1-10		<0.1-5		<0.1-14	Outdoor (c)	

(a) 20 cm above water surface; (b) 150 cm above water surface; (c) 200 cm above water surface.

Table 10. Comparison of concentrations of trihalomethanes (THM) in blood of swimmers after 1 h of exercise, in pool water and in ambient air of indoor and outdoor pools [35].

	THM (mean - range)	
	indoor pools	outdoor pools
Blood of swimmers (µg/l)	0.48(0.23-0.88)	0.11(<0.06-0.21)
Pool water (µg/l)	19.6(4.5-45.8)	73.1(3.2-146)
air 20 cm above water surface (µg/m ³)	93.6(23.9-179.9)	8.2(2.1-13.9)
air 150 cm above water surface (µg/m ³)	61.6(13.4-147.1)	2.5(<0.7-4.7)

Absorptions estimated by us confirmed that the quantity of chloroform taken up by inhalation was a major portion of the total dose for bathers and instructors, being 57% compared to 43% absorbed through the skin. These estimates are based on the results of other studies in the literature, from which we obtained lung retention and skin penetration. They are undoubtedly associated with errors because respiratory dose is greatly affected by lung ventilation which was assumed by us without any precise indications about the real volume of air inhaled and without considering differences between subjects due to physical exertion and age. As far as we are aware, no similar estimates have been reported in the literature and therefore our data forms an excellent basis for further research, including epidemiological studies. The data should be implemented in this way to make it more representative. The good correlation observed with urinary concentrations at the end of exposure and with delta after/before exposure confirms that even if our estimates were not quantitatively exact, they are highly indicative of exposure.

5. CONCLUSIONS

This study shows that concentration of trihalomethanes in pool water vary as a consequence of the type of disinfectants used and of the impurities in the treatment reagents. Trihalomethanes are lost from the surface of the water and are found in the air above the pool. Water-air transport depends on a number of factors that include concentrations in pool water, temperature and water disturbance by bathers. The sampling site and bathing complex significantly affect air concentrations. Absorption of trihalomethanes for workers and swimmers, during time spent at the pool, evaluated by urinary excretion of the same compounds before and after exposure, is higher for subjects carrying on activity in the water, confirming the importance of skin and digestive absorption, although inhalation is on average the major portion of the total absorbed dose.

The results show that even “healthy” places like pools can pose chemical agent management problems that are far from simple. Since the aim of water treatment is to control biological risk for users, it is senseless to categorically condemn swimming pools or water chlorination. “The risks to health from these by-products at the

levels at which they occur in pool water are extremely small in comparison with both the risks associated with inadequate disinfection and the enormous health benefits (including relaxation and exercise) associated with pool use” [1]. It is therefore to be hoped that more attention be paid to the design and management of pools, as well as to correct behaviour, in order to improve our living and occupational environments.

This study considers all the aspects related to the trihalomethanes exposure in indoor swimming pool disinfected with chlorine and derivatives, and the results can be generalized and applied in similar situations.

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Hydroxypropylmethylcellulose gel application delays Der p 1 diffusion in vitro

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ABSTRACT

Background: A special hydroxypropylmethylcellulose powder (Nasaleze®) has been used for the alleviation of nasal symptoms of allergic rhinitis since 1994. The efficacy of the product has been recently proven but the mechanism of action was still largely unknown. The aim of the study was to investigate the hypothesis that the gel formed after moisture absorption in the nose might act as mechanical barrier that prevents allergen diffusion towards the nasal epithelium. **Methods:** The diffusion of Der p 1 through HPMC and agar gels was measured in vitro after 15, 30, 60, 180 and 360 minutes using ELISA. Agar blocks were used to simulate the nasal mucosa. Control samples without gel layer were obtained. **Results:** The control samples with no applied gel barrier absorbed 72.2 % of the Der p 1 solution after 15 minutes and 100 % after 60 minutes. In comparison, the HPMC and agar gel layers both significantly delayed Der p 1 diffusion. After 15 minutes 0.76 % had diffused through the HPMC gel layer compared to 28.1 % which diffused through the agar layer. After 360 minutes, 14.1 % of the baseline Der p 1 crossed the HPMC gel layer while 100 % had diffused through the agar layer. **Conclusions:** HPMC gel significantly reduces Der p 1 diffusion in vitro compared to no barrier and an agar gel layer. This is likely to be due to the small mesh size of the polymer network of HPMC and could have important implications for a preventative treatment of allergic rhinitis.

Keywords: Allergic Rhinitis; Der p 1; Diffusion Barrier; Hydroxypropylmethylcellulose

1. INTRODUCTION

Allergic rhinitis (AR) is a global health problem which

affects up to 25 % of the adult population in industrialised countries and more than 40 % of children [1,2]. The rising prevalence of allergic rhinitis imposes a huge burden on the economy due to costs of treatment and loss of work productivity. Recent estimates of annual costs range from \$2 to 5 billion in the U.S. alone [3-5]. The pathology of AR is associated with a severe impairment of the quality of life for those who suffer from it [6,7]. A reduction of quality-of-life impairment can be achieved by appropriate treatment of allergic rhinitis [7,8]. Modern medications such as antihistamines or corticosteroids can do a lot to help to alleviate symptoms and restore a normal lifestyle but many of them have unwanted adverse effects or are limited in their application [1,3,4]. Many people distrust these conventional medicines and therefore prefer to use complementary and alternative treatments. However, the therapeutic efficacy of many of these treatments is not supported by evidence and they might not be devoid of side effects [3,9].

A recent approach is offered by the use of an inert hydroxypropylmethylcellulose (HPMC) powder (Nasaleze®) for allergy prevention and alleviation in the nose. Although the product has been registered as a class 1 medical device with the MHRA since 1991 and is sold over the counter in more than 50 countries worldwide, little work has been done on the effect of the powder on nasal symptoms. However, the efficacy of HPMC in decreasing symptoms of allergic rhinitis caused by grass pollen and house dust mite allergens was recently proven [10-12]. The investigators observed an improvement of symptoms when using HPMC for treatment of SAR and PAR. Nasal peak inspiratory flow (PIF) and peak expiratory flow (PEF) increased compared to placebo and some symptoms of allergic rhinitis including sneezing, itching and runny nose were alleviated significantly. Also the need to use rescue medication was found to be reduced. Considerable variance was observed in the results and some participants did not show any improvement. This was partly attributed to the application device which is suspected not to deliver constant doses [12,13].

The HPMC powder is applied to the nose using a specially designed dry powder dispenser bottle and forms a gel on the nasal lining by absorbing moisture from the nasal mucosa. It was hypothesised that this gel might act as a mechanical barrier preventing allergens from entering the mucosa [11,12]. However, no investigations on the mechanism of action of HPMC as an allergy treatment have been published as yet leaving the question how an inert cellulose derivative can offer relief to individuals affected by allergic rhinitis unanswered. Similar HPMC powders which also form hydrogels upon contact with liquids are widely used in controlled drug release formulations where they restrict the release of drug molecules through the tablet by serving as a barrier to drug diffusion [14]. Also, high-viscosity HPMC gels have been shown to limit glucose and cholesterol absorption in the gastrointestinal tract by creating a mechanical barrier [15,16]. Thus, it is assumed that HPMC gel might impede the passage of allergens in a similar manner.

The aim of this study was to investigate the possibility that HPMC gel might constitute a mechanical barrier to house dust mite allergen *in vitro* in order to gain information about the mechanism of action of HPMC in the alleviation of symptoms of allergic rhinitis.

2. METHODS

2.1. Materials

Hydroxypropylmethylcellulose powder was supplied by Colorcon Limited, Kent. Der p 1 solution (in house reference, 7.5 µg Der p 1 per millilitre) was provided by Alk-Abello, Madrid.

2.2. Sample Preparation

Preparation of the samples took place in a cleanroom to minimise contamination by dust or allergens. All equipment needed for preparation was washed in isopropyl alcohol (70 %) for sterilisation and dried before each use. Ten ml of agar (1.5 %, prepared with 0.9 % saline solution) were cast into a petri dish. After cooling, small rectangles of equal dimensions (1 x 1 cm) were cut from the agar and then transferred to cleaned slides. Two lines of warm and therefore liquid Vaseline were drawn with a brush from the two edges of one side of the agar block to the edges of the slides to avoid diffusion of allergens through the side of the block (**Figure 1**). The position of the agar was marked on the bottom of the slide and the agar block was covered by a cover slip that sealed the upper surface of the agar. Allergen solution could therefore diffuse into the agar through only one free edge (**Figure 1**).

To test the barrier function of HPMC, a thin layer of

HPMC gel was applied covering the edge of the agar which was used for allergen application. For this, 50 mg of HPMC powder were mixed with 1 ml physiological saline solution (0.9 %) to form a 5 % gel. Immediately after the mixing of the gel, 0.2 ml was applied to the open edge of the agar block using a 1 ml sterile syringe. The initial thickness of the gel layer was measured at 3 standard points. After covering with a cover slip, 20 µl of the allergen solution were applied to the HPMC gel covering the one side of the agar blocks limited by the Vaseline lines.

The slides were incubated at 35°C and 90 % relative humidity to simulate nasal conditions for 15, 30, 60, 180 and 360 minutes. After incubation the thickness of the HPMC layer was again measured. The agar blocks were then carefully removed from the slides and transferred to labelled microtubes containing 0.5 ml PBS-T as elution medium. Samples were shaken on an Autovortex for 20 seconds followed by shaking overnight on a lab shaker. Samples were stored frozen at -20°C.

2.3. Reference and Control Samples

To investigate the difference of diffusion through HPMC and agar, control samples were produced with an additional agar layer of 1.5 mm (average thickness of the HPMC gel layer calculated from measurements of HPMC samples using a digital caliper) to replace the HPMC gel and treated in exactly the same way as the HPMC samples.

Additionally, control samples with no allergen addi-

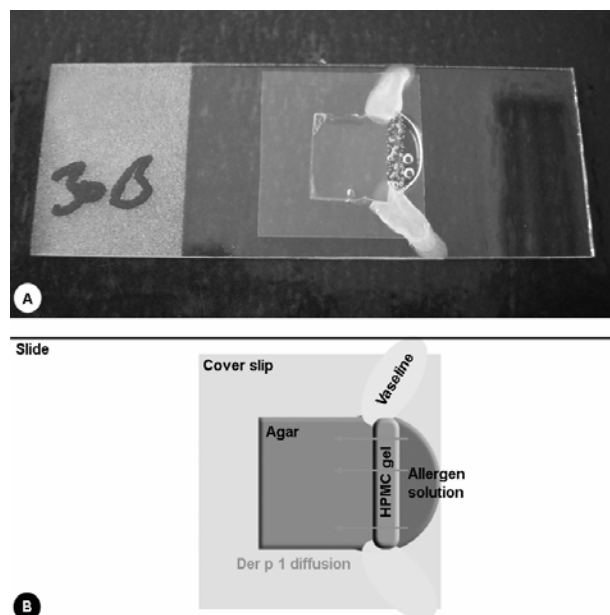


Figure 1. Photograph (A) and diagram (B) of experimental setup for sample preparation for ELISA measurements of Der p 1 diffusion through HPMC gel.

tion and no barrier addition, respectively were obtained.

Baseline measurements of the allergen amount in 20 μ l of allergen solution were conducted by applying 20 μ l of allergen solution directly to a microtube containing 0.5 ml of PBS-T. The microtubes were then treated in the same way as the microtubes containing the agar blocks.

2.4. ELISA Measurements

The monoclonal antibodies (mAbs) and Der p 1 allergen standards used in the assays were purchased from Indoor Biotechnologies, and the assays were performed according to the manufacturer's instructions.

2.5. Statistical Analysis

One-way ANOVA was applied for statistical analysis of the differences between Der p 1 diffusion in HPMC gel, agar gel and control samples, respectively. No serious violations of assumptions were observed. P values of 0.01 or less were considered to be statistically significant.

3. RESULTS

The mean baseline allergen content in 20 μ l of the standard solution used was found to be 151.0 ng/ml (SD = 4.0 ng/ml). This is in good agreement with the calculated value of 150 ng/ml for the given dilution of a 7.5 μ g/ml stock solution. All control samples with no allergen application were negative in the ELISA measurements.

The diffusion of Der p 1 molecules into the 1 x 1 cm agar blocks eluted for measurements was delayed with both gel barriers applied (**Table 1** and **Figure 2**). The amount of allergen diffused through 1.5 mm of 1.5 % agar gel was significantly different from the baseline values for the first 180 minutes ($p < 0.005$) but did not reach statistical significance after 360 minutes ($p = 0.628$). After 15 minutes of incubation, 28.1 % of the baseline allergen amount had diffused through the gel into the agar block (**Table 2**, $p < 0.0001$). The amount of allergen detected in the elutes of the agar blocks then steadily increased until it reached baseline level after 360 minutes of incubation (**Figure 2** and **Table 2**). The thickness of the agar layer applied as a barrier did not change during the measurement times from 15 to 360

minutes. In contrast, an initially 1.50 mm thick HPMC gel layer swelled to an average 3.34 mm in 360 minutes upon allergen solution application. Diffusion of Der p 1 molecules through 5 % HPMC gel showed a significant reduction of diffused allergen for all test times ($p < 0.001$). After 15 minutes 0.76 % of the baseline amount had diffused through the HPMC gel layer into the agar block compared to 28.1 % which diffused through the agar layer (**Table 2**). After 360 minutes, 14.1 % of the baseline Der p 1 crossed the HPMC gel layer while 100 % had diffused through the agar layer (**Table 2**). However, the HPMC data include several outliers and the standard deviation is high (**Table 1**). The mean coefficient of variation for all measurements for the HPMC gel was found to be 201.9 % which is very high compared to 37.8 % for agar.

Control samples with no barrier had absorbed 72.2 % of the baseline allergen content after 15 minutes and differences to baseline did not reach statistical significance after 60 minutes using a 99 % confidence interval ($p_{60\text{min}}=0.042$, $p_{360\text{min}}=0.990$).

4. DISCUSSION

Most of the commonly available treatments of allergic rhinitis affect the inflammatory processes (e.g. by abating mediator release or blocking receptors) initiated after

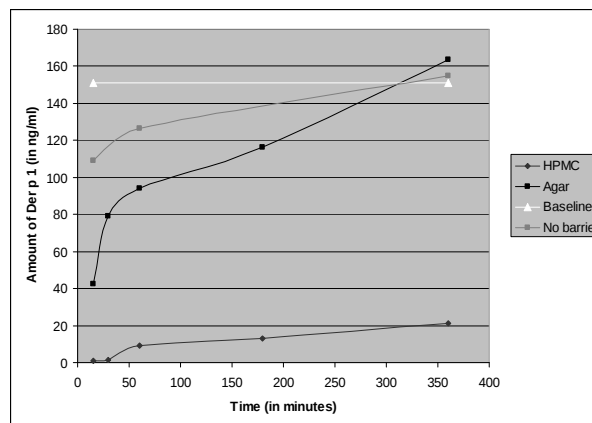


Figure 2. Amount of Der p 1 diffused through a 1.5 mm thick HPMC and agar gel layer, respectively compared to control (no barrier) and baseline allergen amount.

Table 1. Amount of Der p 1 diffused through a 1.5 mm thick HPMC and agar gel layer, respectively, amount of allergen absorbed without barrier (control) and baseline allergen amount in 20 μ l of the applied solution.

Time (in min)	Amount of Der p 1 measured in samples (in ng/ml)				
	15	30	60	180	360
HPMC	1.15	1.57	8.98	13.17	21.34
Agar	42.46	78.98	93.92	116.46	163.59
No barrier	109.26	no value	126.62	no value	154.92
Baseline	151.04	151.04	151.04	151.04	151.04

Table 2. Fractions of allergen amount diffused through a 1.5 mm thick HPMC and agar gel layer, respectively and with no barrier compared to the baseline value of 151.04 ng/ml.

Time (in min)	Diffused fraction of Der p 1 (in % of baseline)				
	15	30	60	180	360
HPMC	0.76	1.04	5.94	8.72	14.13
Agar	28.11	52.29	62.18	77.11	108.31
No barrier	72.34	no value	83.83	no value	102.57
Baseline	100.00	100.00	100.00	100.00	100.00

allergen penetration into the mucosa and binding to IgE [1,17,18] and therefore represent symptomatic treatment. This means that inflammation and the associated damage of the mucosa are already established and the medication decreases signs of this inflammation while it is still on going. An ideal allergy treatment would inhibit the establishment of an allergic reaction altogether. Anti-IgE prevents binding of allergen to IgE antibodies and so inhibits a reaction while the allergens are already inside the epithelium [19]. HPMC might work at an earlier stage by preventing allergens from entering the mucosa in the first place by the generation of a mechanical gel barrier.

The present study aimed to investigate this possible barrier function of HPMC to allergens. The results obtained by ELISA-measurements show that HPMC significantly delays Der p 1 diffusion and that the amount of allergen diffused through the gel is even lower than indicated by preliminary tests [20]. This retardation might allow the mucosa to recover its physical integrity and the allergic reaction to decline. However, a complete barrier to Der p 1 diffusion could not be confirmed.

The retarded diffusion of solutes in hydrogels like HPMC gel or agar gel is well known and widely used for biotechnological separation methods such as electrophoresis or gel chromatography and in controlled release formulations [21,22]. The most comprehensible model developed to explain the diffusion delay of solutes in gels is the obstruction theory which assumes that the impenetrable polymer chains are obstacles that cause an increase in diffusional path length and additionally act as a sieve [21,24]. Therefore the mesh or pore size of the polymer network is a crucial parameter in the reduction of diffusion in hydrogels [25]. Hydrogels consist of high molecular weight molecules forming a three-dimensional network which is dispersed in a continuous liquid medium [22,25]. Due to cross-links and entanglements of these molecules hydrogels can be described as a mesh with solvent filled spaces between the individual polymer chains which act as a filter for molecules larger than the spaces available [26,27]. Controlled release studies with FITC-dextran molecules of different molecular weights revealed that the critical molecular weight for diffusion in HPMC gels, which are characterised by a mesh size of 12 nm, lies between 65 and 66.5 kDa de-

pending on the molecular weight of the polymer and the concentration of the gel [28]. Allergenic proteins usually have a molecular weight between 5 and 80 kDa [29,30]. This means that a great proportion of allergens theoretically are small enough to diffuse through the HPMC mesh spaces. Although Der p 1 (24 kDa) lies well below the mesh size of HPMC gels, a substantial delay in diffusion has been observed. Even though molecules larger than 65 kDa are stopped from diffusing through HPMC almost completely, all other smaller molecules will still be delayed by the longer diffusional path due to obstructions by the macromolecular chains and the slower water movement due to binding of water to the polymer. Furthermore, the mesh size and therefore the size of the spaces available for diffusion in weakly cross-linked homogenous gels is not stable but time-dependent and the size and location of the spaces change due to Brownian motion of the molecule chains [22,31].

In comparison to HPMC, the mesh size of a 1.5 % agar gel as used in this study has been observed to be between 70 and 800 nm [21,26]. Even the lowest of these values is almost six times larger than the mesh size of HPMC which explains the higher allergen diffusivity within agar gel.

The values obtained in the present study are valid for Der p 1 and allergens of the same or very similar molecular weight. It has been shown that the diffusion coefficient for globular proteins in agar decreases with increasing molecular weight and therefore radius of the proteins [21]. This leads to the assumption that allergens smaller than Der p 1 like Bet v 1 (17 kDa) or grass group 2/3 allergens (10-12 kDa) might be expected to diffuse faster whereas larger allergens like Amb a 1 (38-50 kDa) or Art v 1 (28-60 kDa) might exhibit slower diffusion velocities through the HPMC gel network.

The variability of the results of the measurements of Der p 1 diffusion through HPMC gel was high with a coefficient of variation (CV) of just over 200 %. In comparison, the CV of Der p 1 diffusion in agar gel was only about 37 %. For this reason the variation in the amount of allergen diffusing through the HPMC gel layer cannot solely be attributed to limitations in the methods that were applied. Similarly high variability of diffusion coefficients was obtained for mucus gels [32]. This was attributed to the heterogeneous nature of the

mucous gel producing uneven penetration profiles. Release from HPMC matrices for controlled drug release was found to be sensitive to alterations in the chemical composition and the polymer gel conformation and substantial batch-to-batch variations in release and swelling could be observed for a single type of HPMC [33,34]. The authors suspect that this might be due to aggregate formation in the gel causing transient cross-linking that could perturb diffusion in some places throughout the gel which cannot be predicted.

Due to its importance in controlled drug release, the effect of HPMC as a diffusion barrier for drugs has been studied extensively. However, no investigations of allergen diffusion in HPMC have been found in the accessible literature. It was confirmed in this study that HPMC gel delays Der p 1 diffusion in vitro. Other allergens need to be tested to extend the evidence for the efficacy of the product. Also many other factors will influence the efficiency of the product in vivo. For practicality reasons, the gel layer used in the experiments is thicker than the gel layer that can be expected to be established within the nasal cavity. Diffusion velocity is a crucial parameter needed to make assumption for in vivo conditions and should therefore be addressed in future research. A complete diffusion barrier is essential for the retardation of drug release [14] and similarly optimal coverage of the nasal mucosa is important since uncovered areas may allow free allergen entry and the provocation of an allergic response. Sub-optimum coverage is likely to reduce the efficiency of the product. The provision of a suitable powder delivery device therefore poses an important challenge for the maximisation of the efficacy of HPMC in the alleviation of allergic rhinitis.

In conclusion, a diffusion delay of Der p 1 in HPMC gel has been confirmed in vitro. This means that even though HPMC gel does not constitute an impermeable barrier to allergens, the significant delay of allergen entry into the mucosa could be beneficial to hay fever sufferers through the reduction of allergen exposure. This fairly novel way of treatment reduces the allergen load itself and not the symptoms caused after allergen entry into the mucosa. Thus, with the appropriate delivery device, HPMC could be a valuable, drug-free alternative for the treatment of allergic rhinitis. The efficacy of HPMC in hay fever treatment has been recently proven [10-12]. However, the research presented in this paper is the first to address the mechanism of action of HPMC in the alleviation of allergic rhinitis. This knowledge will allow improvements on the product to be made in order to increase its benefit to hay fever sufferers.

5. ACKNOWLEDGEMENTS

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Metal ion-binding properties of L-glutamic acid and L-aspartic acid, a comparative investigation

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ABSTRACT

A comparative research has been developed for acidity and stability constants of $M(\text{Glu})^1$, $M(\text{Asp})^2$ and $M(\text{Ttr})^3$ complexes, which have been determined by potentiometric pH titration. Depending on metal ion-binding properties, vital differences in building complex were observed. The present study indicates that in $M(\text{Ttr})$ complexes, metal ions are arranged to the carboxyl groups, but in $M(\text{Glu})$ and $M(\text{Asp})$, some metal ions are able to build chelate over amine groups. The results mentioned-above demonstrate that for some $M(\text{Glu})$ and $M(\text{Asp})$ complexes, the stability constants are also largely determined by the affinity of metal ions for amine group. This leads to a kind of selectivity of metal ions, and transfers them through building complexes accompanied with glutamate and aspartate. For heavy metal ions, this building complex helps the absorption and filtration of the blood plasma, and consequently, the excursion of heavy metal ions takes place. This is an important method in micro-dialysis. In this study the different aspects of stabilization of metal ion complexes regarding to Irving-Williams sequence have been investigated.

Keywords: Glutamic Acid; Aspartic Acid; Tartaric Acid; Divalent Metal Ions; Potentiometric Titration; Acidity and Stability Constants.

1. INTRODUCTION

It is known that metal ions are important for numerous biochemical reactions. For example, enzymes work only in the presence of such metal ions. The metal ion complexes of many amino acids have been investigated [1-6]. Functionalized membranes represent a field with multiple applications. Examination of specific metal-macromolecule interactions on these surfaces indicates an ex-

¹L-Glutamic acid; ²L-Aspartic acid; ³L-Tartaric acid

cellent method for characterization of these materials. Ion exchange, chelation, and electrostatic interactions form the basis of metal sorption. The behavior of various materials functionalized with polypeptides and other molecules is a topic of interest because of its applications in affinity separations, biosensors, and other applications including site-specific interactions [7]. An example of the latter involves the removal of heavy metals from aqueous solutions [8-11]. These sorbents are made of a variety of materials containing different functional groups. The advantage of affinity separations is that they may be tailored for the desired selectivity and capacity. The functionalization of materials is of vital importance for the production of new materials with specific properties. The characterization of these new materials is also critical. Previous investigations showed that for the “harder” ligands, the ionic term dominates and their binding energies are affected by changes in covalency over the series. It is the competition between these behaviors that produces the Irving-Williams series in stability constants [12]. L-glutamate and L-aspartate are key molecules in cellular metabolism (**Figure 1**). In humans, dietary proteins are broken down by digestion into amino acids, which serve as metabolic fuel for other functional roles in the body. Based on above-mentioned, the essential role of Glu is interesting to study the interaction between other metal ions with Glu and related compounds.

2. EXPERIMENTAL

2.1. Materials

The L-glutamic acid and L-aspartic acid (extra pure) was purchased from Merck, Darmstadt, Germany. The nitrate salt of Na^+ , Ca^{2+} , Mg^{2+} , Mn^{2+} , Co^{2+} , Cu^{2+} , and Zn^{2+} (all pro analysis) were from Merck. All the starting materials were of reagent grade and used without further purification. Potassium hydrogen phthalate and standard solutions of sodium hydroxide (titrasol), nitric acid, EDTA and of the buffer solutions of pH 4.0, 7.0 and 9.0 were all from Merck. All solutions were prepared with deionized water. Water was purified by Milil-Q water purification system, de-ionized and distilled.

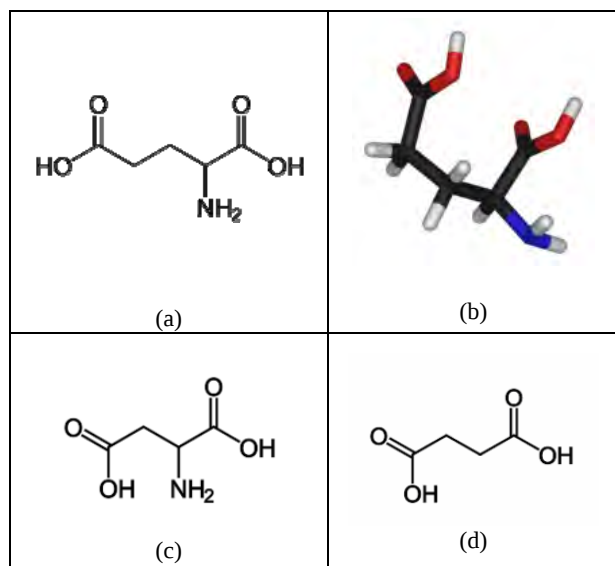


Figure 1. Chemical structures of (a, b) L-glutamic acid; (c) L-aspartic acid and; (d) tartaric acid.

2.2. Ph Titrations

Reagents

Carbonate-free sodium hydroxide 0.03 M was prepared and standardized against sodium hydrogen phthalate and a standard solution of nitric acid 0.5 mM. M(II) nitrate solution (0.03 M) was prepared by dissolving the above substance in water and was standardized with standard solution of EDTA 0.1 M (triplex).

2.3. Apparatus

All pH titrations were performed using a Metrohm 794 basic automatic titrator (Titrino), coupled with a thermostating bath Hero at 25°C ($\pm 0.1^\circ\text{C}$) and a Metrohm combined glass electrode (Ag/AgCl). The pH meter was calibrated with Merck standard buffer solutions (4.0, 7.0 and 9.0).

2.4. Procedure

For the determination of acid dissociation constants of the ligand L, an aqueous solution (0.03 mM) of the protonated ligand was titrated with 0.03 M NaOH at 25°C under nitrogen atmosphere and ionic strength of 0.1 M, NaNO₃. For the determination of binary (a ligand and Cu²⁺) system, the ratios used were 1:1, Cu(II) : Ligand and 1:1, Cu(II) : L, 0.3 mM. This solution was titrated with 0.03 M NaOH under the same conditions mentioned above. Each titration was repeated seven times in order to check the reproducibility of the data.

2.5. Calculation

The acid dissociation constants, $K_{H_2(L)}^H$ and $K_{H(L)}^H$ for

H₂(L) were calculated by an algebraic method. The equilibria involved in the formation of 1:1 complex of L and a divalent metal ion may be expressed as Eqs.(7-10).

3. RESULTS AND DISCUSSION

The potentiometric pH-titrations (25°C, 0.1 M, NaNO₃) were carried out to obtain the acidity and stability constants which are summarized in **Tables 1 and 2**.

Acidity constants

L-glutamate $-\text{O}_2\text{CCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{CO}_2^-$ and L-aspartate ions (L²⁻), $-\text{O}_2\text{CCH}_2\text{CH}(\text{NH}_2)\text{CO}_2^-$, are two-basic species, and thus they can accept two protons, given H₂(L), for which the following de-protonation equilibria are hold:

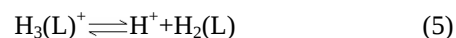


$$K_{H_2(L)}^H = [\text{H}(\text{L})^-][\text{H}^+]/[\text{H}_2(\text{L})] \quad (2)$$



$$K_{H(L)}^H = [\text{L}^{2-}][\text{H}^+]/[\text{H}(\text{L})^-] \quad (4)$$

The two proton in H₂(L) are certainly bound at the terminal acetate and amino groups, i.e., it is released from $\text{HO}_2\text{CCH}_2\text{CH}_2\text{CH}(\text{NH}_3^+)\text{CO}_2^-$ H₂Glu according to Equilibria (1) and (2). It is known as zwitter-ion, and is also closed to the de-protonation of acetate groups which occurs at the terminal acetate groups of tartaric acid [6,13]. L²⁻ can release one more proton from the terminal acetate group. Hence, here due addition to Equilibrium (3) should be considered, which takes place above a pH ≈ 2 (see **Figure 2**).



$$K_{H_3(L)}^H = [\text{H}_2(\text{L})][\text{H}^+]/[\text{H}_3(\text{L})^+] \quad (6)$$

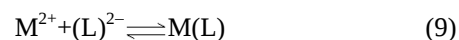
Here, the aforementioned reaction is not considered further.

3.1. Stability of Binary and Ternary Complexes

If we abbreviate for simplicity associating with Ca²⁺, Mg²⁺, Mn²⁺, Co²⁺, Cu²⁺, and Zn²⁺ with M²⁺, then one may write the following two Equilibria (4) and (5):



$$K_{M(\text{H};\text{L})}^M = [\text{M}(\text{H};\text{L})^+]/[\text{M}^{2+}][\text{H}(\text{L})^-] \quad (8)$$



$$K_{M(L)}^M = [\text{M}(\text{L})]/[\text{M}^{2+}][\text{L}^{2-}] \quad (10)$$

3.2. Potentiometric Analyses

These results are summarized in **Tables 1 and 2**. The data

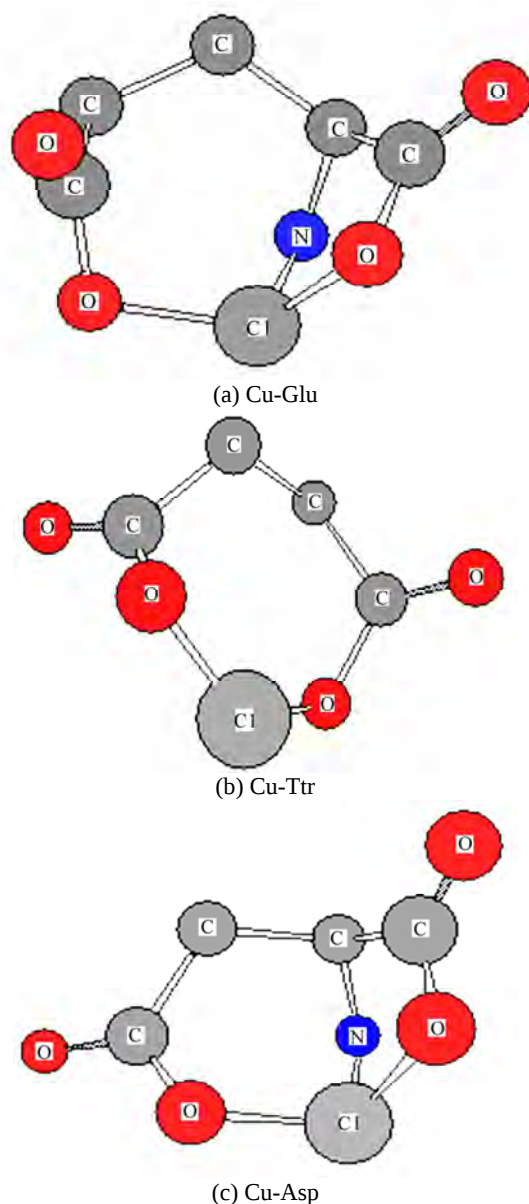


Figure 2. Schematic structures of the species with interactions according to Equilibrium (5) for a) Cu(Glu), b) Cu(Asp) and c) Cu(Ttr). The structure in the right part of the figure was drawn with the program CS Chem 3D, version 3.5, from Cambridge Software Corporation.

Table 1. Negative logarithm of the acidity constants of $H_3(Asp)^+$, $H_2(Ttr)^+$, and $H_3(Glu)^+$ at 25°C, 0.1 M, $NaNO_3^*$, Equilibriums (1), (2) & (3).

	Asp	Ttr	Glu	Site
	1.99**	3.09±0.07	2.05±0.13	-CO ₂ H
pK _a	3.72±0.03	4.19±0.05	4.37±0.03	-CO ₂ H
	9.90±0.03	—	9.98±0.02	-NH ₃

*The given errors are three times the standard error of the mean value or the sum of the probable systematic errors. **[6]

Table 2. Comparison of the stability constants of binary complexes of Asp, Ttr and Glu with M^{2+} at 25°C, $I = 0.1$ M, $NaNO_3^*$.

No.	Species	$\log K_{M(Asp)}^M$	$\log K_{M(Ttr)}^M$	$\log K_{M(Glu)}^M$
1	Mg ²⁺	2.50±0.06	1.90±0.05	1.82±0.06
2	Ca ²⁺	1.26±0.06	1.80±0.05 ¹	1.41±0.02
3	Mn ²⁺	3.91±0.03	4.08±0.08	3.19±0.08
4	Co ²⁺	6.69±0.06	3.27±0.08	4.15±0.09
5	Cu ²⁺	8.78±0.02	3.65±0.07	7.70±0.09
6	Zn ²⁺	5.35±0.06	2.69±0.07	5.84±0.03

*The given errors are three times the standard error of the mean value or the sum of the probable systematic errors. ¹[6,14]

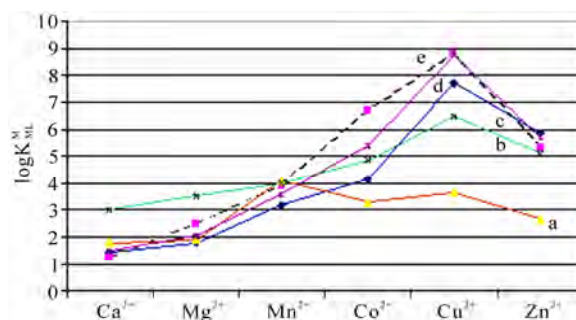


Figure 3. Irving-Williams sequence-type plot for the 1:1 complexes of Ca²⁺, Mg²⁺, Mn²⁺, Co²⁺, Cu²⁺, and Zn²⁺ formed with. a) Tartarate (Ttr²⁻); b) oxalate (Ox²⁻); c) glutamate (Glu²⁻); d) glycine (Gly⁻); e) aspartate (Asp²⁻); The data are taken from Table 2 and [15], they represent also the stability constants of M^{2+} complexes of L (25°C, 0.1 M, $NaNO_3$).

in Table 1 present the acidity constants pK_a ($K_{H(L)}^H$, $K_{H_2(L)}^H$, and $K_{H_3(L)}^H$) of L-aspartic acid, L-tartaric acid, and L-glutamic acid (Eqs.(1-6)). L-aspartic acid can release totally three protons, two protons from carboxyl groups and one from amino group. L-tartaric acid contains no amino group, so two reported acidity constants regard to carboxyl groups. In case of L-glutamic acid two protons are released from carboxyl groups and one proton from amino group, which were showed as regarding sites. As we can see from these results, the stability constants of the binary complexes, such as $M(L)$ (Figure 3) were refined separately using the titration data of this system in a 1:1, ligand: M^{2+} ratio in the same conditions of temperature and ionic strength (according Eqs.(9-10)), as they were in good agreement with reported value [6,14]. We didn't receive reasonable results for $K_{M(H;L)}^M$. The stability constants of

Table 2 show the following trends. The obtained order for Ttr is $Ca^{2+} < Mg^{2+} < Mn^{2+} < Co^{2+} < Cu^{2+} > Zn^{2+}$. The corresponding order for Glu and Asp is $Ca^{2+} < Mg^{2+} < Mn^{2+} < Co^{2+} < Cu^{2+} > Zn^{2+}$. The last observed stability order for Glu and Asp follows the Irving-Williams sequence [15].

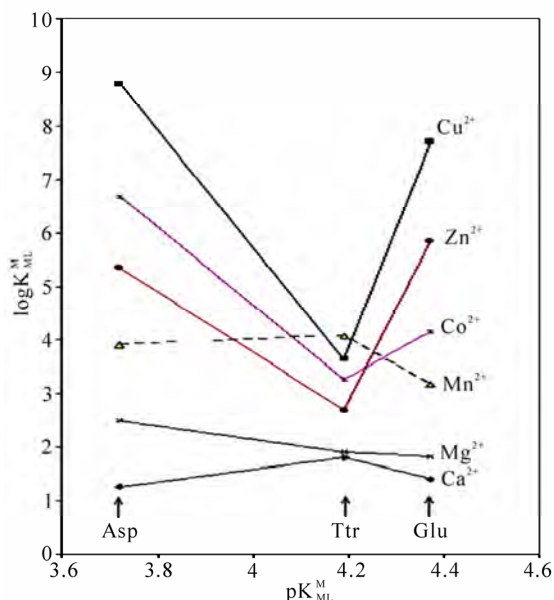


Figure 4. Relationship between $\log K_{ML}^M$ and pK_{HL}^H for Ca^{2+} , Mg^{2+} , Mn^{2+} , Co^{2+} , Cu^{2+} , and Zn^{2+} 1:1 complexes of glutamate (Glu^{2-}), tartarate (Ttr^{2-}) and aspartate (Asp^{2-}). All plotted equilibrium constant values refer to aqueous solutions at 25°C, 0.1 M, $NaNO_3$ (Table 1, second row & Table 2).

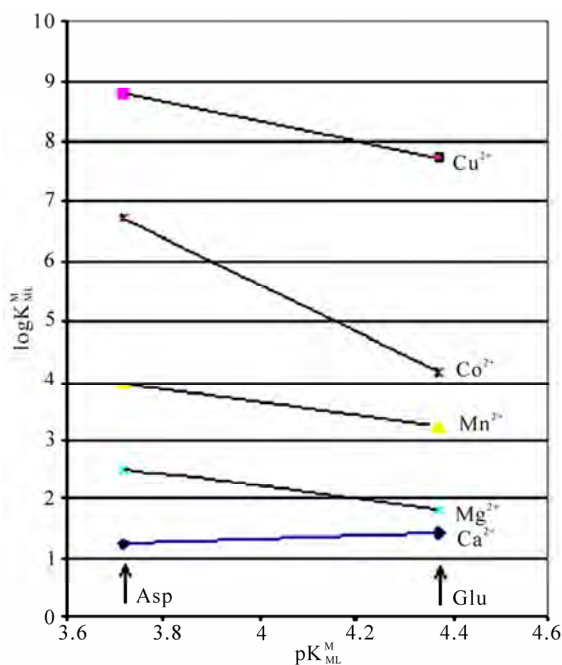


Figure 5. Relationship between $\log K_{ML}^M$ and pK_{HL}^H for Ca^{2+} , Mg^{2+} , Mn^{2+} , Co^{2+} , and Cu^{2+} , 1:1 complexes of glutamate (Glu^{2-}) and aspartate (Asp^{2-}). All plotted equilibrium constant values refer to aqueous solutions at 25°C, 0.1 M, $NaNO_3$ (Table 1, second row & Table 2).

As we can use from **Figure 3**, glutamate and aspartate chelate metal ions weakly via the amino nitrogen and carbonyl oxygen. A stronger chelation occurs upon amide nitrogen bound hydrogen by some metal ions such as Cu^{2+} . This reaction occurs in neutral pH conditions ($pH \approx 7$) with Cu^{2+} . A crystal structure of M^{2+} chelate with a structure analogous has been studied [16].

In **Figure 4**, we can consider the relationships between $\log K_{ML}^M$ and pK_{HL}^H for Ca^{2+} , Mg^{2+} , Mn^{2+} , Co^{2+} , Cu^{2+} , and Zn^{2+} 1:1 complexes of glutamate (Glu^{2-}), aspartate (Asp^{2-}) and tartarate (Ttr^{2-}). The data are taken from **Table 2**. They also represent the stability constant values.

All the plotted equilibrium constant values refer to aqueous solution at 25°C, $I=0$ M ($NaNO_3$). As one can see, this relationship is not linear. The interesting point is that in case of hard metal ions such as Ca^{2+} and Mn^{2+} , the results for Ttr show a maximum $\blacktriangle$ and in case of softer metal ions such as Co^{2+} , Cu^{2+} , and Zn^{2+} , they show a minimum $\blacktriangledown$. This is an indication, that for softer metal ions an additional interaction such as with amino group exists, which we can not find by Ttr.

In **Figure 5**, we can consider the relationships between $\log K_{ML}^M$ and pK_{HL}^H for Ca^{2+} , Mg^{2+} , Mn^{2+} , Co^{2+} , and Cu^{2+} 1:1 complexes of glutamate (Glu^{2-}) and aspartate (Asp^{2-}). However, the most important question is that: Is there a correlation between complex stability and donor groups' basicity? In other words, is there a linear relationship between $\log K_{ML}^M$ and pK_{HL}^H ? Based on **Figure 5**, it seems that this is the case, where the data pairs for the systems of several metal ions are plotted. It is interesting to point out, that in contrast to increasing of basicity from Asp to Glu, show the stability of regarding complexes a decreasing trend.

Now, we are able to compare the stability constants of two species $M(Glu)$ and $M(Asp)$. It could easily distinguish that those constants of $M(Asp)$ are generally larger than those of the corresponding $M(Glu)$ species. This increased stability of the difference between the stability constants as defined in Eq.(11):

$$\Delta \log K = \log K_{(Asp)} - \log K_{(Glu)} \quad (11)$$

Positive amount of $\Delta \log K$ indicates that the $M-Glu$ complexes in case of Mg, Mn and Cu are less stable than $M-Asp$ complexes. These decreases of the stability, from Asp to Glu are based on larger ring size of Glu in $M-Glu$ complexes. The calculated results of $\Delta \log K$ for Mg, Mn and Cu are 0.68, 0.72 and 1.08, respectively.

The standard Gibbs free energy change for the reaction is related to the following equilibrium constant

$$\Delta G^\ominus = -2.303 RT \log K \quad (12)$$

As results, we received the according calculated values for Gibbs free energy change 3.88, 4.11 and 6.16 kJmol^{-1} for Mg, Mn and Cu, respectively. If we compare

these values with the plotted results in **Figure 5**, we distinguish the decrease of stability constants from Asp – to Glu complexes, which refers to weaker interaction between metal ions and regarding ligands. This is a sequence of well-known ring size of the chelate. In case of M-Asp complexes is seven-member chelate ring and in M-Glu complexes is eight-member chelate ring. In case of Ca^{2+} the difference is within error range.

According to Irving and Williams, the order was as a consequence of the fact that the two parameters, which serve as a guide to the magnitude of the ionic (electrostatic) and covalent interactions (the reciprocal of the metal ionic radius and the sum of the first two ionization energies, respectively), both increase monotonically throughout the series from Mn to Cu and then decrease from Cu to Zn. Thus, if water is replaced from $[\text{M}(\text{OH}_2)_n]^{2+}$ by a ligand of better electron-donating power, then the gain in stability will increase with the ionization potential of the metal. If H_2O is replaced by a ligand with a formal negative charge, the stability gain through electrostatic forces will increase as the radius of the metal cation decreases.

A series of metal-varied $[\text{ML}(\text{SC6F5})]$ model complexes related to blue copper proteins have been studied by a combination of absorption [12]. Thus, the “softer” thiolate ligand can have comparable covalent and ionic contributions to bonding, and these compensate to produce little change in the binding energy over the series of metal ions (open squares in **Figure 6**). For the “harder” ligands (F^- , OH^- , H_2O , etc.), the ionic term dominates and their binding energies are affected by changes in covalency over the series (open circles in **Figure 6**). It is the competition between these behaviors that produces the Irving-Williams series (solid circles in **Figure 6**) in stability constants.

Biological systems have the ability to selectively bind to metals taking advantage of the array of protein binding functionalities [17]. Short chain synthetic biopolymers also have unique, strong and selective binding properties offered by their constituent amino acids.

Interactions between aspartic acid (Asp) and cytidine-5-monophosphate (CMP) in metal-free systems as well as the coordination of $\text{Cu}(\text{II})$ ions with the above ligands were studied. The composition and overall stability constants of the species formed in those systems were determined [18]. Amino acid chelated minerals, also referred to as chelated minerals or mineral chelates, are minerals that have been chemically engineered to become more bio-available to our body. Amino acids act as carriers to ship the much-needed minerals to the destination (the small intestine) where consumption takes place.

Elixir Industry has tested many self-claimed “mineral chelates” available on the market and found that most of them are merely mixtures of amino acids and inorganic

minerals [19]. Why are amino acid chelated minerals superior to common inorganic minerals? Chelated minerals help protect vitamin stability. Inorganic metal ions may serve as a catalyst to further the oxidation and degradation of vitamins. Chelated minerals, on the other hand, are well shielded by bonded organic ligands, which we can consider in **Figure 3**. They will not come in contact with vitamin molecules; thus, the vitamins will be protected from oxidation and degradation. And since magma precipitation is prevented, chelated minerals will not absorb vitamins and cause them to become non-absorbable, problems that common inorganic mineral are known to cause. As we can see, in **Figure 3**, Ttr is not able to build three dentate chelate like Asp, so that metal ions are not enough shielded. As consequence metal ions can take part in substitution reactions.

There are different types of amino acids. L-aspartic acid plays a vital role in energy production and is a major excitatory neurotransmitter. It is involved in building DNA (genetic structures in cells), in carbohydrate metabolism & protein metabolism. It helps detoxify ammonia in the body, helps reduce fatigue and depression, and it also supports liver protection. Mineral Chelates (for example AbSolu) are chelates of L-aspartic acid and one of the minerals such as calcium, magnesium and zinc.

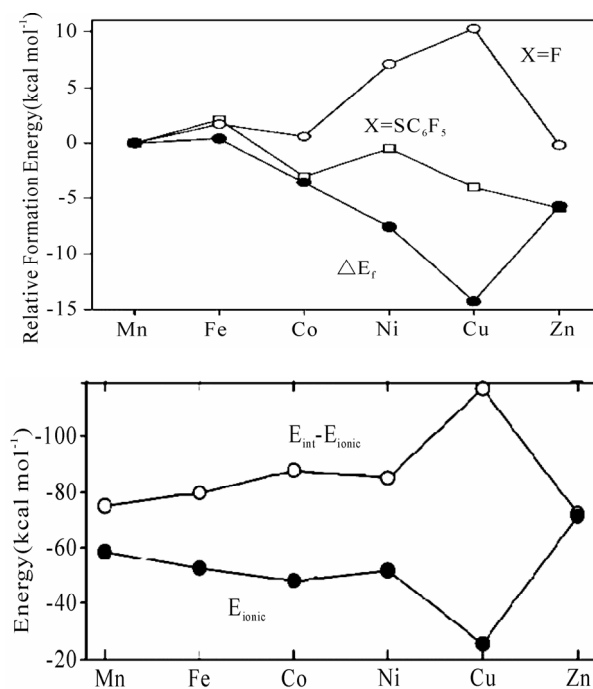


Figure 6. (a) Relative formation energies (kcal mol^{-1}) of the metal-thiolate complexes, $[\text{ML}(\text{SC6F5})]$, and the metal-fluoride complexes, $[\text{ML}(\text{F})]$, and their difference, ΔE_f ; (b) Ionic component of the $\text{ML}^+-\text{SC6F5}$ - bonding energy, E_{ionic} , and the difference, $E_{\text{int}} - E_{\text{ionic}}$. Edward I. Solomon *et al.*, Inorg. Chem. 44 (2005) 4947-4960.

There are different types of amino acids. Nutrition scientists selected L-aspartic acid based on many additional benefits that come with it. Most of the competitors' products contain two or more crystalline water in their molecules. Theoretically those products should not be referred to as "chelates" and the bonding (if indeed exists) of amino acid molecules to mineral ions is vulnerable. These products are anhydrous chelates of two L-aspartic acid molecules and a single metal ion. Based on the results of this work, we can draw the conclusion, that hard metal ions just with identical stability constants could have similar interaction with glutamate. Even based on these, results of acidity constants reported (**Table 2**) glutamic acid occurs in high organism in form of glutamate. Earlier works have reported the structure of glutamate complexes with some metal ions [20] such as Co^{2+} , Cu^{2+} and Zn^{2+} . These metal ions are able to have additional interactions with glutamate. This leads to building of macro-chelate (**Figure 3**).

4. CONCLUSIONS

It is shown, that Glu has identical complex properties as Asp i.e. the both amino acids have similar structure. The only difference is also the size of amino acids. The previous studies have shown, that Glu has special applications [21].

The glutamate industry have found that manufactured free glutamic acid, in the form of monosodium glutamate (MSG), hydrolyzed vegetable proteins, etc., etc., when added to the processed foods, masks off flavors and makes the blandest and cheapest foods taste wonderful. An other interesting point is the pharmacological application of new generation of glutamate and aspartate complexes and it seems essential to understand their reaction mechanisms.

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A longitudinal study of the professional dispositions of teacher candidates

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ABSTRACT

The purpose of this study was to examine the professional disposition scores of Physical Education teacher candidates over time. In addition, differences between teacher and student ratings were investigated. Participants were 65 students who completed three methods courses (A, B, and C) across a two-year period. Both the teacher and the students completed a professional dispositions instrument in each of the three classes. Results indicated a decrease in disposition self ratings and teacher ratings over time. A 2 (Rater) x 3 (Time) ANOVA revealed that the student and teacher ratings were different for classes A and B but not for class C. The findings are encouraging in light of the strong alignment between teacher and student ratings in the upper-level class. The dispositions instrument appears to be a valid and reliable method to assess the professional behaviors of teacher candidates.

Keywords: Dispositions; Teacher Education; Professional Behavior

1. INTRODUCTION

Both the National Council for the Accreditation of Teacher Education (NCATE) and the Interstate New Teacher Assessment and Support Consortium (INTASC) now mandate the assessment of dispositions in teacher education programs. Specifically, the NCATE 2000 Standards require schools, colleges and departments of education to use performance-based evidence to demonstrate that teacher candidates in all programs are gaining the knowledge, skills and dispositions necessary to have a positive impact on K-12 student learning. Similarly, the INTASC standards emphasize knowledge, skills, and dispositions that highlight the importance of performance-based assessments. More recently, the National Initial Physical Education Teacher Education Standards

(2008) added a standard requiring the assessment of professional dispositions. Standard number 6 states that teacher candidates must demonstrate dispositions that are essential to becoming effective professionals. Accordingly, accredited teacher education programs throughout the nation have incorporated professional dispositions, in addition to the preexisting knowledge and skill performance requirements for prospective teacher candidates.

The assessment of professional behaviors has created considerable discussion and controversy among teacher education programs and teaching professionals. Most teaching professionals acknowledge the difficult challenges in attempting to quantify personality characteristics and qualities [1,2]. Of great concern and scrutiny are the specifics of “how,” “who,” and “when” of dispositions. For instance, issues relating to the origins of dispositions, the appropriate context for assessment, and the purposes they serve are all under question and examination. One of these challenges is the considerable variability in the methods used to assess dispositions. Behavioral checklists, observations, reflections, journals/logs, video analyses, portfolios, and rating scale rubrics are all examples of how dispositions are assessed. Ideally, instruments should offer the student the opportunity to reflect upon strengths, weaknesses, and teaching philosophy, as well as provide professional faculty the occasion to provide constructive feedback to the student [2]. Equally important is the opportunity for teacher candidates to develop a clear understanding of self and students [3].

Although the “who” of disposition assessment may seem obvious (teacher education majors), an important point of concern is the equally important variable of “who” conducts the assessment. Feedback is typically gathered from a combination of the following sources: the teacher candidates; instructors, program coordinators, field placement supervisors, and mentor teachers [1,2,4]. Even though gathering information from a pool of teachers seems beneficial, this raises concern as to how to best balance evaluation procedures among students and teachers. Relying on a combination of student

self-report measures and professional faculty assessments seems to provide the most reliable appraisal.

An additional issue relating to dispositional assessment is “when” it should occur. The timeline varies from state to state and from institution to institution. In most cases, disposition assessment occurs at several stages in a variety of settings throughout the teacher preparation curriculum [1,4,5]. In some cases though, dispositions may only be addressed in the event of concerns being expressed about a teacher candidate as more of a remediation measure [2]. Most researchers agree that the assessment of professional dispositions must occur early and often in the professional preparation program.

There are those who are critical of efforts to gauge the dispositions of pre-service teachers and of attempts to influence the development of dispositions as part of teacher education training [6]. It is clear, however, that assessing teacher dispositions has taken a foothold in teacher education programs and will only continue to be more fully integrated into the undergraduate curriculum. In comparison to skill performance and knowledge assessment instruments, there are only a few instruments to assess professional dispositions. Perhaps more importantly, few studies have investigated changes in the professional dispositions of teacher candidates over time. In other words, what impact does the teacher education program have on a teacher candidate’s professional behavior?

The purpose of this study was to examine the professional disposition scores of Physical Education Teacher Education (PETE) students over time. In addition, differences between student and teacher ratings were investigated.

2. METHOD

2.1. Participants

The participants for this longitudinal study were 65 PETE major students who completed three methods courses representing a 3-semester sequence. The same university instructor taught each of the three classes.

2.2. Instrument

A Professional Dispositions Assessment that was developed and tested at a large Midwestern university was used for this study. This assessment contains the following 10 items identified by the PETE faculty as representative of professional behavior:

- 1) Attendance
- 2) In class performance
- 3) Class preparation
- 4) Relationship with others
- 5) Group work
- 6) Professional development

- 7) Respect for school rules, policies, norms
- 8) Emotional control/responsibility
- 9) Role model
- 10) Communication

For each item, there are four possible ratings representing unsatisfactory, basic, proficient, and distinguished behavior (see [7] for a more detailed explanation of the instrument).

2.3. Procedure

Professional dispositions are assessed by the instructor in seven PETE classes during the 4-year program. In most classes, students are required to complete a self assessment of their professional dispositions. One of the PETE requirements is to achieve a basic or higher rating for all ten items in each of the seven classes. If a student receives an unsatisfactory rating, she/he must complete a formal remediation process prior to enrolling in another PETE class.

The three classes examined in this study represent 2nd and 3rd-year courses in the major. Prior to enrolling in the first of these three classes, students receive a thorough explanation about the dispositions instrument in two first-year classes. In addition, they complete a self assessment and receive an assessment from their teachers.

The first class (A) is typically taken during the 3rd semester in the program. This class is an introduction to teaching physical education course and includes 12 hours of assisting/teaching in local schools. The second class (B) is a motor development class and includes 10 hours of teaching preschool students. The third class (C) is an Elementary School Methods class and includes a 15-hour practicum in a local school. Due to pre-requisites, the 3 courses are taken sequentially over a 3-semester period.

In each of the three classes, the participants were evaluated by the instructor during the final week of the semester. In addition, they completed and submitted a self assessment during this same week. Although the instructor included the dispositions evaluation as part of the course grade, the relative point values differed across the three classes. For the purpose of comparison, the disposition ratings for each of the three classes were converted to percentage scores.

2.4. Data Analyses

A 2 (Rater) X 3 (Time) ANOVA was used to analyze the disposition data. To keep the risk of a Type I error low, the alpha level was established at $p < 0.01$ so that only results with probabilities of sampling errors of less than 1 in 100 would be declared statistically significant.

3. RESULTS

Descriptive statistics showed the means for student rat-

ings by time point were 94 (A), 94 (B), and 84 (C), respectively. The means for teacher ratings were 89 (A), 85 (B), and 82 (C), respectively. An additional finding was that five participants received at least one “unsatisfactory” rating from the instructor.

As shown in **Figure 1**, the student ratings were similar in classes A and B but dramatically decreased from class B to class C. Student ratings in class C were significantly lower ($p=0.0001$) than in A and B. For teacher ratings, there was a descending linear trend across the three time periods. Teacher ratings were significantly different between classes A and C only ($p=0.0001$).

The 2 X 3 ANOVA indicated that the student and teacher ratings were significantly different for classes A ($p=0.005$) and B ($p=0.001$) with the teacher ratings lower than those of the students. Differences between student and teacher ratings for class C, however, did not reach significance.

4. DISCUSSION

An interesting finding was the decrease in disposition self ratings and teacher ratings over time. Although the students rated themselves similarly in the first two classes, their self ratings dropped considerably in the upper-level class. It should be noted that this decrease in disposition scores does not necessarily reflect a decline in professional behavior. Expectations increase as students progress through the program. For example, the expectations for a 1st or 2nd-year student in the area of professional development would be less than the expectations for a 3rd or 4th-year student. Consequently, a student in class A may receive a “proficient” rating for attending two professional conferences (in one year) while a student in class C might receive a “basic” rating.

The significant decline in self ratings from class A and B to class C may be attributed to an improvement in self awareness. In fact, students in class C are re-

quired to reflect upon their strengths and weaknesses after each teaching episode. They specifically focus on organization/management, instruction, and professional behavior. In addition, they receive specific feedback through coding analyses. Using this feedback, they set specific goals for the next lesson. It is likely that these opportunities to reflect on specific behaviors provide students with an increasingly clear and realistic self-portrait.

A comparison of the student and teacher ratings indicates that the ratings were most aligned in class C. In fact, the student and teacher ratings did not significantly differ in the final class. Because students tended to inflate their disposition scores in the first two classes, their scores were significantly higher than those of the teacher. The considerable drop in self ratings in class C was probably a result of critically reflecting on professional behaviors.

A final finding was that none of the five students who received an “unsatisfactory” rating participated in the formal remediation program. Each of the students dropped out of the PETE program by choice. It should be noted that these students also failed to meet at least one other PETE requirement (e.g., GPA, PRAXIS I, Portfolio review).

The findings of this preliminary study are encouraging in light of the strong alignment between student and teacher ratings in the upper-level class. It appears that the instrument is a reliable and valid method to assess the professional dispositions of pre-service teachers. It would be interesting to further investigate the efficacy of the instrument by examining additional variables such as GPA and retention. Although the results of this study are preliminary, the findings suggest that the Professional Dispositions instrument is a viable solution to the challenge of assessing the professional behaviors of teacher education candidates.

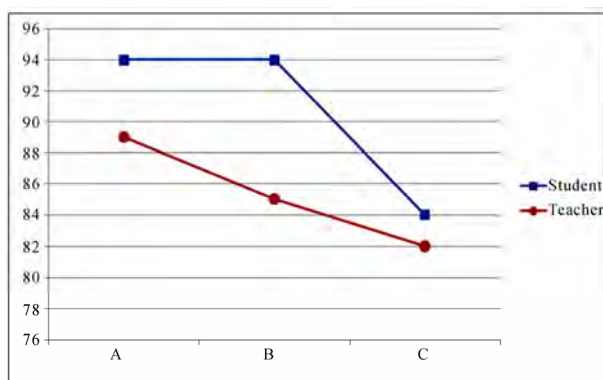


Figure 1. Disposition scores by rater.

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Magnetohydrodynamic equations for toroidal plasmas

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ABSTRACT

A set of reduced MHD equations is derived using the standard energy balance equation. By applying assumption of internal energy, i.e. $R^2u = \text{const}$, a set of reduced magnetohydrodynamic equations are obtained for large aspect ratio, high β tokamaks. These equations include all terms of the same or der as the toroidal effect and only involve three variables, namely the flux, stream function and internal energy.

Keywords: Magnetohydrodynamics; Aspect Ratio; Viscosity

1. INTRODUCTION

Magnetohydrodynamic (MHD) instabilities [1], such as tearing modes, play an important role in plasma behaviour of tokamaks. They may influence both the particle and the energy confinement and are considered to be one of the main reasons for disruptions. Hence they have been studied by many authors [2-4]. The method of reduced magnetohydrodynamic based on a large-aspect ratio expansion has provided a powerful method for linear and nonlinear numerical computations.

In previous studies [2-4], the density of plasma is assumed to be constant. This assumption is valid in cylindrical geometry because the divergence of velocity is of the order ε^3 with ε being the ratio of the minor radius to the length of cylinder. Thus we can neglect the divergence of velocity in the continuity equation. By applying assumption of density, i.e. $R^2\rho = \text{const}$, Ren *et al.* [5] has derived the reduced MHD Equations in toroidal geometry for large aspect ratio, low β tokamaks. Since the divergence of velocity is of the order ε^2 in toroidal geometry the same order as the toroidal effect, and cannot be neglected. Hence the assumption of constant density does not agree satisfactorily with the continuity equation in toroidal geometry. The present paper extends this work to high β tokamaks.

In this paper, by using a new assumption about internal energy that is, $R^2u = \text{const}$ a set of reduced MHD Equations is derived. Where R is major radius and u is an internal energy. The reduced MHD Equations involve three variables: the flux, stream function and internal energy. The Equations can be used to calculate the nonlinear evolution of tearing modes for toroidal plasmas.

2. REDUCED MHD EQUATIONS

The basic MHD Equations are of the form

$$\frac{3}{2} \frac{dP}{dt} + \frac{5}{2} P \nabla \cdot \mathbf{v} = 0 \quad (1)$$

$$\rho \frac{\partial \mathbf{v}}{\partial t} = -\nabla P + \mathbf{J} \times \mathbf{B} \quad (2)$$

$$\mathbf{J} = \nabla \times \mathbf{B} \quad (3)$$

$$\frac{\partial \mathbf{B}}{\partial t} = -\nabla \times \mathbf{E} \quad (4)$$

$$\mathbf{E} + \mathbf{v} \times \mathbf{B} = \eta \mathbf{J} \quad (5)$$

In MHD, if $u = \frac{P}{\gamma - 1}$, denote internal energy than standard energy Balance Equation has the form

$$\frac{du}{dt} + \gamma u \nabla \cdot \mathbf{v} = 0 \quad (6)$$

For the sake of simplicity, we adopt the quasi-cylindrical coordinate (r, θ, φ) with φ being the toroidal angle. By assuming the inverse aspect ratio ε to be a small quantity, the ordering of the high β tokamaks [4].

$$O(1) = B_\varphi, \frac{\partial}{\partial r}, \frac{1}{r} \frac{\partial}{\partial \varphi}$$

$$O(\varepsilon) = B_\perp, J_\varphi, v_\perp, \frac{\partial}{\partial t}, \frac{1}{R}, u$$

$$O(\varepsilon^2) = \tilde{B}_\varphi, J_\perp, v_\varphi$$

where $B_\phi = \frac{B_0 R_0}{R}$ is the externally applied magnetic field with R_0 being the coordinate of the geometric center of plasma and $B_0 = B_\phi|_{R=R_0}$. $\tilde{B}_\phi$ is the toroidal magnetic field produced by plasma current. J_ϕ is the toroidal current and v_ϕ the toroidal velocity. The subscript $\perp$ denotes perpendicular to $\nabla\phi$. The u is the internal energy assumed to be of the order ε , for high β tokamaks. For simplicity we use B_ϕ to express RB_ϕ , $\tilde{B}_\phi$ to $R\tilde{B}_\phi$, J_ϕ to RJ_ϕ , v_ϕ to Rv_ϕ . Thus B_ϕ is of the order ε^{-1} , J_ϕ is of zeroth order while $\tilde{B}_\phi$ and v_ϕ are of the order ε . Furthermore we assume η to be of the order ε^2 , for high β tokamaks [4].

Introducing the vector potential A for magnetic field B , that is,

$$A = A_r e_r + A_\theta e_\theta + A \nabla\phi \quad (7)$$

Then B can be expressed as

$$= \nabla A \times \nabla\phi - \frac{1}{R} \left(\frac{\partial A_\theta}{\partial\phi} e_r - \frac{\partial A_r}{\partial\phi} e_\theta \right) + \left(B_\phi - \frac{R}{r} \frac{\partial A_r}{\partial\phi} + R \frac{\partial A_\theta}{\partial r} \right) \nabla\phi \quad (8)$$

From **Eq.(8)** one can see that A is of zeroth order and A_r and A_θ are of the order ε^2 . Compared with equilibrium magnetic field expressed as $B = \nabla\psi \times \nabla\phi + (B_\phi + \tilde{B}_\phi) \nabla\phi$, where A is the flux function. Later it will be shown that the effect of A_r and A_θ is of the order of ε^2 and can be neglected so that within our approximation B can be determined by A only. Substituting **Eqs.(8)** and **(5)** into **Eq.(4)** we obtain

$$\nabla \times \frac{\partial A}{\partial t} = \nabla \times (\nu \times B) - \eta \nabla \times J, \quad \frac{\partial A}{\partial t} = (\nu \times B) - \eta J + \nabla\phi \quad (9)$$

where ϕ is the gauge potential. **Eq.(9)** can be deduced as

$$-\frac{B_\phi}{R^2} \nu_\perp + \nabla\phi \times \nabla\phi = O(\varepsilon^4)$$

Hence we can introduce stream function U for ν

$$U = \frac{\phi}{B_0}, \quad (10)$$

where U is the order ε and ν can be expressed as

$$\nu = \frac{R^2}{R_0} \nabla U \times \nabla\phi + \nu_\phi \nabla\phi + O(\varepsilon^3) \quad (11)$$

where the relation $B_\phi = B_0 R_0$ is used. The divergence

of ν is obtained as

$$\nabla \cdot \nu = \frac{1}{R_0} \nabla R^2 \times \nabla U \cdot \nabla\phi + O(\varepsilon^3). \quad (12)$$

This **Eq.(12)** shows that the divergence of ν is of the order ε^2 . Taking the ϕ component of **Eq.(9)** and substituting **Eqs.(10)** and **(12)** into **Eq.(9)**, we obtain the flux evolution equation

$$\frac{\partial A}{\partial t} = \frac{R^2}{R_0} B \cdot \nabla U + O(\varepsilon^2). \quad (13)$$

Taking the curl of B , we get the expression of current

$$J_\phi = R^2 \nabla\phi \cdot (\nabla \times B) = -R^2 \nabla \cdot (R^{-2} \nabla A) + O(\varepsilon^2) \quad (14)$$

where the ϕ -derivatives in operator are of the higher order and can be dropped. From the internal energy evolution **Eq.(6)** by substituting **Eq.(11)**, we obtain

$$\frac{d}{dt} (R^2 u) = O(\varepsilon) \quad (15)$$

The term on the right-hand side of **Eq.(15)** can be neglected because the term on the left-hand side is of zeroth order. This indicates that if we assume the internal energy to be constant, **Eq.(15)** cannot be satisfied. We assume

$$R^2 u = R_0^2 u_0 = \text{const} \quad (16)$$

This assumption satisfies **Eq.(15)** and includes the toroidal effect.

Taking the curl of momentum **Eq.(2)** after multiplying by R^2 we can eliminate $J_\perp$ and B_ϕ . Then we get

$$\rho_0 \frac{dW}{dt} = -\frac{R^2}{R_0} B \cdot \nabla J_\phi + (\gamma - 1) \frac{R^2}{R_0} \nabla R^2 \times \nabla u \cdot \nabla\phi + O(\varepsilon^2) \quad (17)$$

where

$$W = R^2 \nabla^2 U,$$

$$\frac{d}{dt} \approx \frac{\partial}{\partial t} + \frac{R^2}{R_0} \nabla U \times \nabla\phi \cdot \nabla.$$

The ϕ -derivatives in the Laplacian are also of the higher order and can be neglected. Using the Assumption of **Eq.(16)** we can directly obtain the parallel viscosity equation. Then a set of reduced MHD equations can be written as

$$\frac{\partial A}{\partial t} = \frac{R^2}{R_0} B \cdot \nabla U \quad (18)$$

$$\rho_0 \frac{dW}{dt} = -\frac{R^2}{R_0} B \cdot \nabla J_\phi + (\gamma - 1) \frac{R^2}{R_0} \nabla R^2 \times \nabla u \cdot \nabla\phi \quad (19)$$

$$\frac{d}{dt} (R^2 u) = 0 \quad (20)$$

$$J_\phi = -R^2 \nabla \cdot (R^{-2} \nabla A) \quad (21)$$

$$W = R^2 \nabla^2 U, \quad (22)$$

The Assumption 16 that the product of the square of the major radius and the internal energy is a constant, is quite stringent as taking this to be a constant, the first driving term in the Grad-Shafranov equation becomes just proportional to the flux derivative of the logarithm of the major radius, which shows a rather weak dependence. Therefore, this assumption seems to drastically narrow down the range of equilibrium configurations to which it is applicable. On the other hand, it has been observed [6] that a high density region appears near the inside limiter, which means that the density profile at the inside and outside of plasma along a flux surface is asymmetric. The pressure is calculated as the product of experimental temperature and density. Since the internal energy is related to the pressure as $u = \frac{P}{\gamma - 1}$, we can say

that the pressure distribution is nonuniform poloidally and the pressure is higher at the inside of plasma than at the outside. The result is, however, consistent with our expectation. On the other hand, density and pressure profile widths are clearly correlated [7].

3. CONCLUSIONS

In summary, we derived the reduced MHD Equations (18-22) by using the Assumption 16 about the internal energy in a large aspect ratio limit. These equations in

clude all terms of the same order as the toroidal effect and only involve three variables, namely the flux, stream function and internal energy. These equations can be used to investigate the time evolution of tearing mode for the high β , large aspect ratio limit for tokamak Plasmas.

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Research on the nonlinear spherical percolation model with quadratic pressure gradient and its percolation characteristics

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ABSTRACT

For bottom water reservoir and the reservoir with a thick oil formation, there exists partial penetration completion well and when the well produces the oil flow in the porous media takes on spherical percolation. The nonlinear spherical flow equation with the quadratic gradient term is deduced in detail based on the mass conservation principle, and then it is found that the linear percolation is the approximation and simplification of nonlinear percolation. The nonlinear spherical percolation physical and mathematical model under different external boundaries is established, considering the effect of wellbore storage. By variable substitution, the flow equation is linearized, then the Laplace space analytic solution under different external boundaries is obtained and the real space solution is also gotten by use of the numerical inversion, so the pressure and the pressure derivative bi-logarithmic nonlinear spherical percolation type curves are drawn up at last. The characteristics of the nonlinear spherical percolation are analyzed, and it is found that the new nonlinear percolation type curves are evidently different from linear percolation type curves in shape and characteristics, the pressure curve and pressure derivative curve of nonlinear percolation deviate from those of linear percolation. The theoretical offset of the pressure and the pressure derivative between the linear and the nonlinear solution are analyzed, and it is also found that the influence of the quadratic pressure gradient is very distinct, especially for the low permeability and heavy oil reservoirs. The influence of the non-linear term upon the spreading of pressure is very distinct on the process of

percolation, and the nonlinear percolation law stands for the actual oil percolation law in reservoir, therefore the research on nonlinear percolation theory should be strengthened and reinforced.

Keywords: Nonlinear Spherical Percolation; Quadratic Pressure Gradient; Percolation Characteristics; Reservoir; Partial Penetration Completion Well; Mathematic Model

1. INTRODUCTION

So far, the research on nonlinear percolation has increasingly aroused widespread concern and attention. The nonlinear percolation is the modern development of a new direction [1]. Retaining the nonlinear term was proposed by Odeh A S [2]. He thought ignoring the quadratic gradient term would cause larger error in hydraulic fracturing, big pressure drop flow, DST and large pressure drop pulse testing. Bai M Q [3] considered that ignoring the quadratic gradient term in flow equation is equivalent to ignoring convection flow term in diffusion-convection equation. Wang Y [4] established the nonlinear flow model in poroelastic media. Chakrabarty C [5] derived the mathematical model with nonlinear diffusion equation and made the quantitative analysis of the quadratic term. Braeuning S [6] established the nonlinear radial flow model of the variable-rate well-test. Tong Dengke [7,8] solved the well test models of heterogeneous and dual porosity reservoir. Concerning the spherical flow, the linear spherical flow model was studied by William E. Brigham, Charles A. Kohlhaas and Mark A. Proett, *et al.* [9-11]. In their models, no nonlinear spherical flow model is found, so this paper presents the nonlinear spherical flow model and researches its percolation characteristics for partial penetration completion well in the formation.

2. DEDUCTION OF THE NONLINEAR SPHERICAL PERCOLATION EQUATION

When single-phase fluid flow through porous medium, it would conform to the mass conservation principle, so by this principle the flow equation of continuity can be expressed by

$$\frac{\partial}{\partial x}(\rho v_x) + \frac{\partial}{\partial y}(\rho v_y) + \frac{\partial}{\partial z}(\rho v_z) = -\frac{\partial}{\partial t}(\rho\phi) \quad (1)$$

where: v is flow velocity, cm/s; ρ is oil density, g/cm³; t is flow time, s; ϕ is rock porosity, fraction; x, y, z represent the Cartesian coordinates.

If ignoring the impact of gravity and capillary forces, and the inertial resistance is not considered, it would conform to the Darcy's law, so the equation of motion is as follows

$$v = -\frac{k}{\mu} \nabla p \quad (2)$$

where: k is rock permeability, μm^2 ; μ is fluid viscosity, mPa·s; p is formation pressure, MPa.

The fluid flow through porous medium is a process of percolation, and is also a state of constantly changing process, in which the parameters related to percolation are constantly changing with pressure and temperature. Usually the change of temperature in reservoir is inappreciable, so the flow is taken as isothermal flow. The rock and fluid are elastic and slightly compressible, the state equation of fluid and the state equation of rock are expressed as follows respectively

$$\rho = \rho_0 e^{C_p(p-p_0)} \quad (3)$$

$$\phi = \phi_0 e^{C_f(p-p_0)} \quad (4)$$

where: C_p is oil compressibility, MPa⁻¹; C_f is rock compressibility, MPa⁻¹; the subscript "0" represents reference value, usually use the value in standard conditions.

Substitute Eq.(2) into Eq.(1)

$$\frac{\partial}{\partial x}(\rho \frac{k_x}{\mu} \frac{\partial p}{\partial x}) + \frac{\partial}{\partial y}(\rho \frac{k_y}{\mu} \frac{\partial p}{\partial y}) + \frac{\partial}{\partial z}(\rho \frac{k_z}{\mu} \frac{\partial p}{\partial z}) = \frac{\partial}{\partial t}(\rho\phi) \quad (5)$$

$$\frac{\partial}{\partial x}(\rho \frac{k_x}{\mu} \frac{\partial p}{\partial x}) = \frac{\rho k_x}{\mu} \frac{\partial^2 p}{\partial x^2} + \frac{\rho}{\mu} \frac{\partial p}{\partial x} \frac{\partial k_x}{\partial x} + \frac{k_x}{\mu} \frac{\partial p}{\partial x} \frac{\partial \rho}{\partial x} \quad (6)$$

Changing the form of Eq.(3)

$$p = \frac{1}{C_p} \ln \rho - \frac{1}{C_p} \ln \rho_0 + p_0 \quad (7)$$

$$\frac{\partial p}{\partial x} = \frac{1}{\rho C_p} \frac{\partial \rho}{\partial x} \quad (8)$$

$$\frac{\partial p}{\partial t} = \frac{1}{\rho C_p} \frac{\partial \rho}{\partial t} \quad (9)$$

Substitute Eq.(8) into Eq.(6)

$$\frac{\partial}{\partial x}(\rho \frac{k_x}{\mu} \frac{\partial p}{\partial x}) = \frac{\rho k_x}{\mu} \frac{\partial^2 p}{\partial x^2} + \frac{\rho}{\mu} \frac{\partial p}{\partial x} \frac{\partial k_x}{\partial x} + \frac{k_x \rho C_p}{\mu} (\frac{\partial p}{\partial x})^2 \quad (10)$$

By the same method, the following two equations can be deduced

$$\frac{\partial}{\partial y}(\rho \frac{k_y}{\mu} \frac{\partial p}{\partial y}) = \frac{\rho k_y}{\mu} \frac{\partial^2 p}{\partial y^2} + \frac{\rho}{\mu} \frac{\partial p}{\partial y} \frac{\partial k_y}{\partial y} + \frac{k_y \rho C_p}{\mu} (\frac{\partial p}{\partial y})^2 \quad (11)$$

$$\frac{\partial}{\partial z}(\rho \frac{k_z}{\mu} \frac{\partial p}{\partial z}) = \frac{\rho k_z}{\mu} \frac{\partial^2 p}{\partial z^2} + \frac{\rho}{\mu} \frac{\partial p}{\partial z} \frac{\partial k_z}{\partial z} + \frac{k_z \rho C_p}{\mu} (\frac{\partial p}{\partial z})^2 \quad (12)$$

Changing the form of Eq.(4)

$$p = \frac{1}{C_f} \ln \phi - \frac{1}{C_f} \ln \phi_0 + p_0 \quad (13)$$

$$\frac{\partial p}{\partial t} = \frac{1}{\phi C_f} \frac{\partial \phi}{\partial t} \quad (14)$$

Substitute Eq.(14) and Eq.(9) into Eq.(5), the right of Eq.(5) can be changed

$$\frac{\partial}{\partial t}(\rho\phi) = \phi \frac{\partial \rho}{\partial t} + \rho \frac{\partial \phi}{\partial t} = \rho\phi C_p + \rho\phi C_f \frac{\partial p}{\partial t} \quad (15)$$

$$C_t = C_p + C_f \quad (16)$$

where: C_t is total compressibility of rock and oil, MPa⁻¹.

Substitute Eqs.(10)-(12) and Eq.(15) into Eq.(5), we have

$$(k_x \frac{\partial^2 p}{\partial x^2} + k_y \frac{\partial^2 p}{\partial y^2} + k_z \frac{\partial^2 p}{\partial z^2}) + (\frac{\partial p}{\partial x} \frac{\partial k_x}{\partial x} + \frac{\partial p}{\partial y} \frac{\partial k_y}{\partial y} + \frac{\partial p}{\partial z} \frac{\partial k_z}{\partial z}) + C_p [k_x (\frac{\partial p}{\partial x})^2 + k_y (\frac{\partial p}{\partial y})^2 + k_z (\frac{\partial p}{\partial z})^2] + C_f \rho \phi (\frac{\partial p}{\partial t})^2$$

$$k_y \left(\frac{\partial p}{\partial y} \right)^2 + k_z \left(\frac{\partial p}{\partial z} \right)^2 = \mu \phi C_t \frac{\partial p}{\partial t} \quad (17)$$

If the permeability is isotropic and constant, $\partial k_x / \partial r = 0, \partial k_y / \partial r = 0, \partial k_z / \partial r = 0$, the Eq.(17) becomes

$$\left(\frac{\partial^2 p}{\partial x^2} + \frac{\partial^2 p}{\partial y^2} + \frac{\partial^2 p}{\partial z^2} \right) + C_p \left[\left(\frac{\partial p}{\partial x} \right)^2 + \left(\frac{\partial p}{\partial y} \right)^2 + \left(\frac{\partial p}{\partial z} \right)^2 \right] = \frac{\mu \phi C_t}{k} \frac{\partial p}{\partial t} \quad (18)$$

Eq.(18) is the governing differential equation in Cartesian coordinates, the equation in radial spherical coordinates becomes

$$\frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial p}{\partial r} \right) + C_p \left(\frac{\partial p}{\partial r} \right)^2 = \frac{\mu \phi C_t}{k} \frac{\partial p}{\partial t} \quad (19)$$

where, r represents the radial spherical coordinates.

Eq.(19) is the nonlinear flow governing partial differential equation with quadratic pressure gradient term. We call the second power of the pressure gradient as quadratic pressure gradient.

The function $\exp(x)$ by use of Maclaurin series expansion is written by

$$\exp(x) = 1 + x + x^2 / 2 + \dots + x^n / n! + \dots \quad (20)$$

If we use Maclaurin series expansion for Eqs.(3) and (4) and neglect the second order and the above higher order item, the Eqs.(3) and (4) can be rewritten by Eqs. (21) and (22) respectively

$$\rho = \rho_0 [1 + C_p (p - p_0)] \quad (21)$$

$$\phi = \phi_0 [1 + C_t (p - p_0)] \quad (22)$$

The appearance of quadratic pressure gradient term is simply because that we didn't make any simplification for the state Eqs. (3) and (4) in the deduction of the flow governing partial differential equation. If we use Eqs. (21) and (22), instead of Eqs.(3) and (4), in the deduction of the flow governing partial differential equation, the quadratic pressure gradient term will not come up, and the deduced flow equation is the conventional linear flow equation, which is shown in almost any percolation mechanics books and papers, so the deduction of the linear flow equation is certainly omitted here. Owing to the existence of quadratic pressure gradient, the flow equation takes on nonlinear properties. Therefore it can be safely concluded that the conventional linear flow equation is the approximation and simplification of nonlinear flow equation with quadratic pressure gradient term, and that the nonlinear percolation law stands for the actual flow law of oil in reservoir.

3 .SPHERICAL PERCOLATION MODELS AND ITS SOLUTION

3.1. Physical Model

For bottom water reservoir, the position of drilling and completion of oil well is usually in the top of the oil formation, the flow diagram shown in **Figure 1**. For some reservoirs, the oil formation is very thick, the position of drilling and completion of well is usually in the middle of the formation, the flow diagram shown in **Figure 2**. For the two actual situations, the oil flow in the porous media is in the form of spherical percolation.

Physical model assumptions are as follows:

- 1) A single well with partial penetration completion in the formation like **Figure 1** or **Figure 2** products at constant rate, the external boundary may be infinite or closed or constant pressure;
- 2) The rock and the single-phase fluid are slightly compressible, a constant compressibility;
- 3) Isothermal and Darcy flow, the permeability and porosity of isotropic properties;

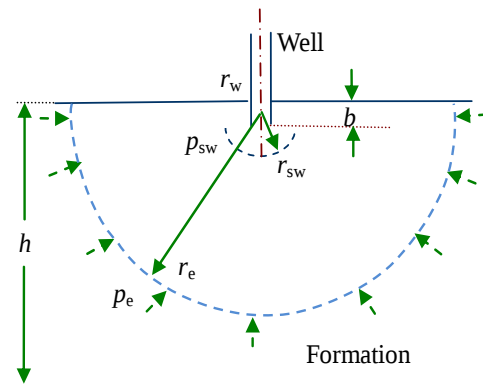


Figure 1. Spherical flow diagram for well completion position in the top of the formation.

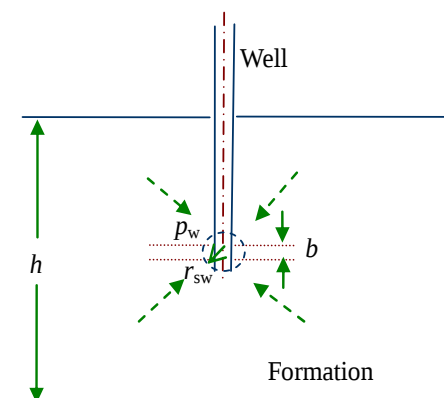


Figure 2. Spherical flow diagram for well completion position in the middle of the formation.

4) Considering wellbore storage effects (in the beginning of opening well, the fluid stored in the wellbore starts to flow, the oil in the formation does not flow);

5) At time $t=0$, pressure is uniformly distributed in the reservoir, equal to the initial pressure p_i ;

6) Ignoring the impact of gravity and capillary forces.

3.2. Mathematic Model

The governing differential equation in radial spherical coordinate system

$$\frac{1}{r^2} \frac{\partial}{\partial r} (r^2 \frac{\partial p}{\partial r}) + C_p (\frac{\partial p}{\partial r})^2 = \frac{\mu \phi C_t}{3.6k} \frac{\partial p}{\partial t} \quad (23)$$

where: r is the radial spherical distance from well, m; the unit of well production time (t) becomes "h", so the coefficient "3.6" appears in the Eq.(23).

Initial conditions

$$p|_{t=0} = p_i \quad (24)$$

where, p_i is initial formation pressure, MPa.

Inner boundary condition

$$\frac{k}{\mu} (r^2 \frac{\partial p}{\partial r})|_{r=r_{sw}} = 0.921 \times 10^{-3} qB + 0.022105 C_s \frac{dp_w}{dt} \quad (25)$$

where: the r_{ws} is called the pseudo well radius of radial spherical flow, and $r_{ws} = b / (2 \ln(b/r_w))$ [12], b is the formation penetration thickness of well completion, m; r_w is the real well radius m; q is oil rate at wellhead, m³/d; B is oil volume factor, dimensionless; C_s is wellbore storage coefficient, m³/MPa; p_w is wellbore pressure, MPa.

External boundary condition

$$\lim_{r \rightarrow \infty} p = p_i \text{ (infinite)} \quad (26)$$

$$p|_{r=r_e} = p_i \text{ (constant pressure)} \quad (27)$$

$$\frac{\partial p}{\partial r}|_{r=r_e} = 0 \text{ (closed)} \quad (28)$$

where, r_e is external boundary radius, m.

3.3. Solution to Mathematic Model

The dimensionless definitions are as follows:

Dimensionless pressure

$$p_D = k r_{sw} (p_i - p) / (0.921 \times 10^{-3} \mu q B);$$

Dimensionless radius based on pseudo spherical flow radius $r_D = r / r_{sw}$;

Dimensionless wellbore storage coefficient

$$C_D = C_s / (3.1416 \phi C_t r_{sw}^3);$$

Dimensionless time $t_D = 3.6kt / (\phi \mu C_t r_{sw}^2)$;

Dimensionless quadratic pressure gradient coefficient

$$\beta = 0.921 \times 10^{-3} qB \mu C_p / (k r_{sw})$$

The dimensionless model is as follows:

The governing differential equation in radial spherical coordinate system

$$\frac{\partial^2 p_D}{\partial r_D^2} + \frac{2}{r_D} \frac{\partial p_D}{\partial r_D} - \beta (\frac{\partial p_D}{\partial r_D})^2 = \frac{\partial p_D}{\partial t_D} \quad (29)$$

Initial conditions

$$p_D|_{t_D=0} = 0 \quad (30)$$

Inner boundary condition

$$C_D \frac{dp_{wD}}{dt_D} - (r_D \frac{\partial p_D}{\partial r_D})|_{r_D=1} = 1 \quad (31)$$

External boundary condition

$$\lim_{r_D \rightarrow \infty} p_D(r_D, t_D) = 0 \text{ (infinite)} \quad (32)$$

$$p_D|_{r_D=r_{eD}} = 0 \text{ (constant pressure)} \quad (33)$$

$$\frac{\partial p_D}{\partial r_D}|_{r_D=r_{eD}} = 0 \text{ (closed)} \quad (34)$$

Take

$$p_D = -\frac{1}{\beta} \ln x \quad (35)$$

where, x is substitution variable between variables.

Making the upper variable substitutions for Eqs. (29-34), the model can be converted to

The governing differential equation

$$(\frac{\partial^2 x}{\partial r_D^2} + \frac{2}{r_D} \frac{\partial x}{\partial r_D}) = \frac{\partial x}{\partial t_D} \quad (36)$$

Initial conditions

$$x|_{t_D=0} = 1 \quad (37)$$

Inner boundary condition

$$(C_D \frac{\partial x}{\partial t_D} - \frac{\partial x}{\partial r_D} + \beta x)|_{r_D=1} = 0 \quad (38)$$

External boundary condition

$$\lim_{r_D \rightarrow \infty} x(r_D, t_D) = 1 \text{ (infinite)} \quad (39)$$

$$x|_{r_D=r_{eD}} = 1 \text{ (constant pressure)} \quad (40)$$

$$\frac{\partial x}{\partial r_D}|_{r_D=r_{eD}} = 0 \text{ (closed)} \quad (41)$$

Take

$$x = y / r_D + 1 \quad (42)$$

where, y is substitution variable between variables.

Making the upper variable substitutions for **Eqs. (36-41)**, the model can be converted to

The governing differential equation

$$\frac{\partial^2 y}{\partial r_D^2} = \frac{\partial y}{\partial t_D} \quad (43)$$

Initial conditions

$$y|_{t_D=0} = 0 \quad (44)$$

Inner boundary condition

$$\left[C_D \frac{\partial y}{\partial t_D} - \frac{\partial y}{\partial r_D} + (\beta + 1)y \right]_{r_D=1} = -\beta \quad (45)$$

External boundary condition

$$\lim_{r_D \rightarrow \infty} y = 0 \text{ (infinite)} \quad (46)$$

$$y|_{r_D=r_{eD}} = 0 \text{ (constant pressure)} \quad (47)$$

$$\left(\frac{\partial y}{\partial r_D} - y \frac{1}{r_D} \right)_{r_D=r_{eD}} = 0 \text{ (closed)} \quad (48)$$

Introduce the Laplace transform based on t_D , that is

$$L[p_D(t_D)] = \bar{p}_D(z) = \int_0^\infty p_D(t_D) e^{-zt_D} dt_D \quad (49)$$

where, z is Laplace space variable.

So, making the Laplace transform of **Eqs.(43-48)**, the model becomes:

The governing differential equation in Laplace space

$$\frac{d^2 \bar{y}}{dr_D^2} - z \bar{y} = 0 \quad (50)$$

Inner boundary condition in Laplace space

$$[(C_D z + \beta + 1)\bar{y} - \frac{d\bar{y}}{dr_D}]_{r_D=1} = -\frac{\beta}{z} \quad (51)$$

External boundary condition in Laplace space

$$\lim_{r_D \rightarrow \infty} \bar{y} = 0 \text{ (infinite)} \quad (52)$$

$$\bar{y}|_{r_D=r_{eD}} = 0 \text{ (constant pressure)} \quad (53)$$

$$\left(\frac{\partial \bar{y}}{\partial r_D} - \bar{y} \frac{1}{r_D} \right)_{r_D=r_{eD}} = 0 \text{ (closed)} \quad (54)$$

The general solution of **Eq.(50)** can be expressed by

$$\bar{y} = A e^{\sqrt{z} r_D} + B e^{-\sqrt{z} r_D} \quad (55)$$

For infinite boundary:

Substitute **Eq.(55)** into **Eq.(52)**, have

$$A = 0 \quad (56)$$

So the general solution of **Eq.(50)** becomes

$$\bar{y} = B e^{-\sqrt{z} r_D} \quad (57)$$

Substitute **Eq.(57)** into **Eq.(51)**, have

$$B = -\frac{\beta}{z(C_D z + \beta + 1 + \sqrt{z}) e^{-\sqrt{z}}} \quad (58)$$

The general solution of **Eq.(50)** can be got by

$$\bar{y} = -\frac{\beta}{z(C_D z + \beta + 1 + \sqrt{z}) e^{-\sqrt{z}}} e^{-\sqrt{z} r_D} \quad (59)$$

At the wellbore bottom, $r=r_w$, $r_D=1$, $p=p_w$, $p_D=p_{wD}$, $x=x_w$, $y=y_w$, therefore, the solution of the spherical percolation model with infinite external boundary in Laplace space can be got by

$$\bar{y}_w = \bar{y}|_{r_D=1} = -\frac{\beta}{z(C_D z + \beta + 1 + \sqrt{z})} \quad (60)$$

The real space solution y_w and the derivative (dy_w/dt_D) can be easily obtained by use of Stehfest numerical inversion [13] for **Eq.(60)**. Substitute the values of inversion into variable substitution relationships, **Eq.(35)** and **Eq.(42)**, so the real space solution p_{wD} and the derivative (dp_{wD}/dt_D) can be certainly gained. Accordingly, the pressure and the pressure derivative bi-logarithmic type curves of nonlinear spherical percolation can be drawn up (see **Figure 3**).

For constant pressure boundary:

At the wellbore bottom $r_D=1$, $y=y_w$, the **Eq.(55)** becomes

$$e^{\sqrt{z}} \cdot A + e^{-\sqrt{z}} \cdot B - \bar{y}_w = 0 \quad (61)$$

Substitute **Eq.(61)** into **Eq.(51)** and **Eq.(53)**, have respectively

$$\begin{aligned} -\sqrt{z} e^{\sqrt{z}} \cdot A + \sqrt{z} e^{-\sqrt{z}} \cdot B + \\ (C_D z + \beta + 1) \bar{y}_w = -\frac{\beta}{z} \end{aligned} \quad (62)$$

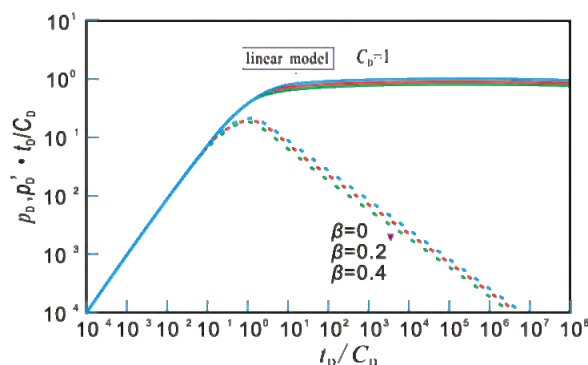


Figure 3. Type curves of nonlinear spherical percolation affected by β under infinite external boundary.

$$e^{\sqrt{z}r_{eD}} \cdot A + e^{-\sqrt{z}r_{eD}} \cdot B = 0 \quad (63)$$

For closed boundary:

Substitute Eq.(61) into Eq.(54), have

$$(\sqrt{z}r_{eD} - 1)e^{\sqrt{z}r_{eD}} A - (\sqrt{z}r_{eD} + 1)e^{-\sqrt{z}r_{eD}} B = 0 \quad (64)$$

Combining Eqs.(61-64), the coefficients A and B and the function at wellbore $\bar{y}_w$ in Laplace space can be easily obtained by use of some linear algebra method (such as Gauss-Jordan reduction, etc), then nonlinear spherical percolation type curves can also be drawn up (see Figure 4 and Figure 5) by use of the same method.

4. CHARACTERISTICS OF THE NONLINEAR PERCOLATION

4.1. Parameter Sensitivity Analysis to Type Curves

Figure 3 shows the type curves of nonlinear spherical percolation affected by β under infinite external boundary. Can be seen from the figure, the curves vary with the value of the dimensionless quadratic pressure gradient coefficient β (from up to down, $\beta=0, 0.2, 0.4$), when $\beta=0$ it is just the curve of linear percolation model. It can be easily seen that the curves have the trait of unit slope in the wellbore storage stage, which shows that there is no influence of quadratic pressure gradient in this flow stage, and that the location of the pressure and the pressure derivative curves is lower than that of the conventional linear model curve in the stage of infinite-acting radial spherical flow. The bigger the β is, the greater the offset is.

Figure 4 and Figure 5 show the type curves of nonlinear spherical percolation affected by β under constant pressure external boundary and closed external boundary respectively. Can be seen from the figures, the trait of unit slope in the wellbore storage stage still exist and there still exists a offset due to the effect of β , but in the late flow stage of boundary response the pressure derivative curves is going down until focusing on a point for constant pressure boundary and the pressure derivative curves is going up until focusing on a line together with the pressure curves for closed boundary, which is completely different from Figure 1.

Figure 6 shows the type curves of nonlinear spherical percolation affected by C_D under infinite external boundary. Can be seen from the figure, the curves vary with the value of the dimensionless wellbore storage coefficient C_D , and the bigger the C_D is, the lower the pressure derivative curve is. Figure 7 shows the type curves of nonlinear spherical percolation affected by r_{eD} under different external boundaries. Can be seen from the figure, the curves vary with the value of the dimensionless radial spherical radius r_{eD} , and the bigger the r_{eD} is, the later the time of going up or going down is.

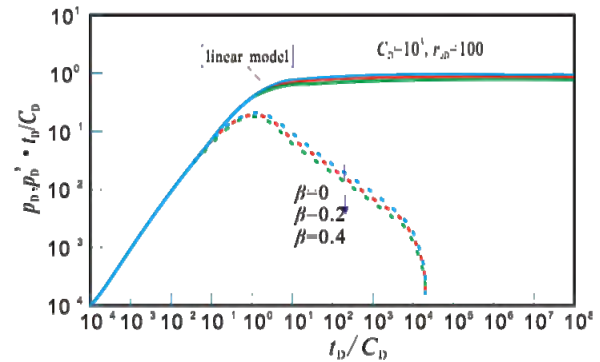


Figure 4. Type curves of nonlinear spherical percolation affected by β under constant pressure external boundary.

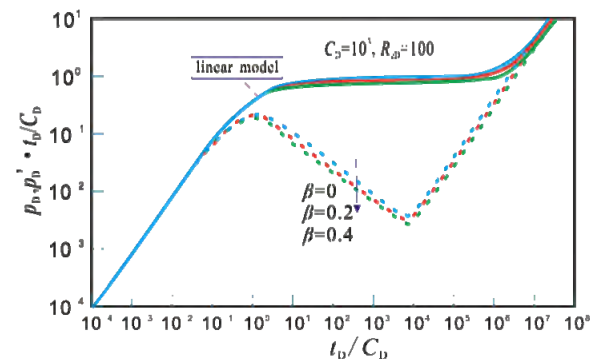


Figure 5. Type curves of nonlinear spherical percolation affected by β under closed external boundary.

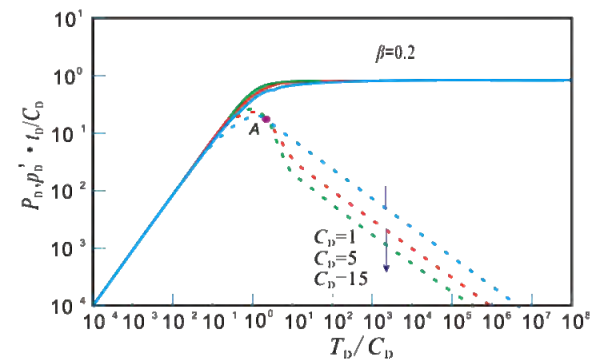


Figure 6. Type curves of nonlinear spherical percolation affected by C_D .

dimensionless radial spherical radius r_{eD} , and the bigger the r_{eD} is, the later the time of going up or going down is.

According to the definition of β and the probable values of β (Table 1), it is clearly demonstrated that β is proportional to oil viscosity μ , and inversely proportional to formation permeability k . So there is usually a bigger β for the low permeability, heavy oil reservoirs, and the influence of the quadratic pressure gradient nonlinear term is very distinct, the quadratic pressure gradient should not be neglected. For the fixed group of parameters (q, B, C_D), the speed of pressure wave propagation

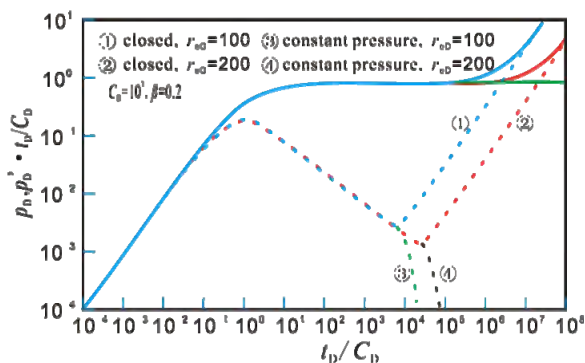


Figure 7. Type curves of nonlinear spherical percolation affected by r_{eD} .

Table 1. The probable values of β .

$k/(\times 10^{-3} \mu\text{m}^2)$	$\mu/(\text{mPa}\cdot\text{s})$	β
100	25	0.0058
10	25	0.0580
1	25	0.5800
100	100	0.0230
10	100	0.2300
1	100	2.3000

becomes slower when k/μ decreases with the increasing of β . Compared with the conventional linear model, given a fixed production time, pressure decline slows down and the speed of decline is inversely proportional to β , which is completely accordant with the theoretical

curves as **Figures 3-5**. In conclusion, for a concrete reservoir, due to the effect of quadratic pressure gradient, compared with conventional linear model, the time of stable production of the nonlinear model is prolonged on condition that the same decline of reservoir pressure.

4.2. The Influence Analysis of Nonlinear Term

Table 2 and **Table 3** exhibit the results of the pressure offset and pressure derivative offset analyses vs. **Figure 3**. As shown the data in these tables, the offset increase with the increasing of time at a constant β , and pressure derivative relative offset is greater than the pressure relative offset at a fixed time. From the data in the table, it is found that the impact of the quadratic gradient is extremely intense when time is particularly long and the quadratic coefficient β is particularly large, so the quadratic pressure gradient should be retained in flow equation. After all, the nonlinear percolation law is the actual flow law of oil in porous medium, so the research on the nonlinear percolation model and its percolation law with quadratic pressure gradient should be strengthened and reinforced.

5. CONCLUSIONS

In this paper it is demonstrated that the quadratic pressure gradient has some distinct influence on the wellbore1) The linear percolation is the approximation and simplification of nonlinear percolation with quadratic pressure gradient term.

Table 2. The offset analysis of nonlinear term($\beta=0.2$).

t_D/C_D	P_{wD}		offset	relative offset /%	$P'_{wD} \cdot t_D / C_D$		offset	relative offset /%
	linear	nonlinear			linear	nonlinear		
10^2	0.943292	0.864578	0.078714	8.34	0.029071	0.024059	0.005012	17.24
10^3	0.982148	0.896753	0.085395	8.69	0.008939	0.007390	0.001549	17.33
10^4	0.994357	0.906908	0.087449	8.79	0.002804	0.002312	0.000492	17.55
10^5	0.998216	0.910121	0.088095	8.83	0.000886	0.000728	0.000158	17.83

Table 3. The offset analysis of nonlinear term($\beta=0.4$).

t_D/C_D	P_{wD}		offset	relative offset /%	$P'_{wD} \cdot t_D / C_D$		offset	relative offset /%
	linear	nonlinear			linear	nonlinear		
10^2	0.943292	0.801013	0.142279	15.08	0.029071	0.020499	0.008572	29.49
10^3	0.982148	0.828462	0.153686	15.65	0.008939	0.006302	0.002637	29.50
10^4	0.994357	0.837153	0.157204	15.81	0.002804	0.001970	0.000834	29.74
10^5	0.998216	0.839906	0.158310	15.86	0.000886	0.000622	0.000264	29.80

2) The new-style type curves of nonlinear spherical percolation with quadratic pressure gradient effect in shape and characteristics are obviously different from the type curves of linear model, the location of the pressure and the pressure derivative curves is lower than that of the conventional linear model curve.

3) The type curves are affected by the quadratic gradient coefficient β , the offset of pressure and pressure derivative is directly proportional to β and time.

4) For a concrete reservoir, due to the effect of quadratic pressure gradient, compared with conventional linear model, the time of stable production of the nonlinear model is prolonged on condition that the same decline of reservoir pressure.

5) The impact of the quadratic pressure gradient under certain conditions is extremely intense, especially for the low permeability and heavy oil reservoirs, and the quadratic pressure gradient term should not be neglected and should be retained in flow equation.

6) The nonlinear percolation law is the actual flow law of oil in porous medium, so the research on the nonlinear flow model and its application with quadratic pressure gradient should be strengthened and reinforced.

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Optical nonlinearity in measurement of urea and uric acid in blood

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ABSTRACT

The Z-scan technique is a simple and effective tool for determining nonlinear optical properties of materials. This technique is utilized in measurement of urea and uric acid in blood. The nonlinear refractive index of urea and uric acid are found to vary linearly with concentration. Hence by calculating the nonlinear refractive index it is possible to measure their concentration in the sample. The results of this method are found to be in good agreement with the conventional colorimetric method.

Keywords: Z-Scan Technique;
Nonlinear Refractive Index; Urea; Uric Acid

1. INTRODUCTION

Urea and Uric acid are the metabolic nitrogenous wastes present in the body that can be measured in blood and urine. Serum uric acid reflects the interactions of four major processes: dietary purine intake, endogenous purine metabolism, urinary urate excretion, and intestinal uricolysis. Uric acid is a metabolite of purines, nucleic acids and nucleoproteins. Consequently, abnormal levels may be indicative of a disorder in the metabolism of these substances. Hyperuricaemia may be observed in renal dysfunction, gout, leukemia, polycythaemia, atherosclerosis, diabetes, hypothyroidism, or in some genetic diseases. Decreased levels are present in patients with Wilson's disease and Fanconis syndrome [1]. The existing methods could be conveniently divided into two groups: reductive and enzymatic. The reductive methods are non-specific and involve the oxidation of uric acid with phosphotungstate reagent to allantoin with resultant blue coloring of tungstate solution.

The enzymatic methods are specific. They involve the catalytic oxidation of uric acid with the enzyme uricase to allantoin with the formation of hydrogen peroxide [2]. The peroxide concentration which is directly proportional to the concentration of uric acid could then be

determined by a number of methods [3-11]. The amount of urea nitrogen is a breakdown product of protein metabolism. Urea formed in the liver as the end product of protein metabolism enters in to the blood and is ultimately eliminated in the urine by the kidneys. Most kidney diseases affect urea excretion so that blood urea nitrogen (BUN) levels increase in the blood. It may also increase the dehydration or bleeding in the stomach and/or intestines or result in any side effect of some medications. Raised levels may also be seen in any state causing hypovolemia, heart failure, starvation and urinary tract obstruction, etc. Urea is one of the first substances to be determined in biological fluids as one of the parameters of liver function tests. In routine procedures urea determination in biological fluids is carried out with chemical reagents and enzymatic methods [12,13].

The Z-scan technique was extending to study the optical nonlinearity of vitreous humor in human and rabbit [14], retinal derivatives [15]. In this present investigation enzymatic reacted urea and uric acid blood sample are subjected to the Z-scan technique to calculate the nonlinear refractive index (n_2). Already work has been done on measurement of glucose [16], total cholesterol and triglycerides [17].

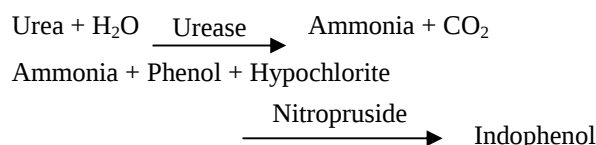
The single beam Z-scan analysis, which was developed by Mansoor Sheik Bahae *et al.* [18], is a simple and effective tool for determining nonlinear optical properties of materials [19-22]. Nonlinear refractive index is proportional to the real part of the third-order susceptibility $\text{Re}[\chi^{(3)}]$. Basically, the Z-scan method consists in translating a nonlinear sample through the focal plane of a tightly focused Gaussian laser beam and monitoring the changes in the far field intensity pattern. For a purely refractive nonlinearity, the light field induces an intensity dependent nonlinear phase and, as consequence of the transverse Gaussian intensity profile, the sample presents a lens-like behavior. The induced self-phase modulation has the tendency of defocusing or re-collimating the incident beam, depending on its Z position with respect to the focal plane. By monitoring the transmittance change through a small circular aperture placed

at the far field position, it is possible to determine the nonlinear refractive index. In this present study, we have measured urea and uric acid levels in blood by calculating the nonlinear refractive index (n_2) value using a single beam Z-scan method.

2. EXPERIMENTAL

2.1. Urea Sample Preparation

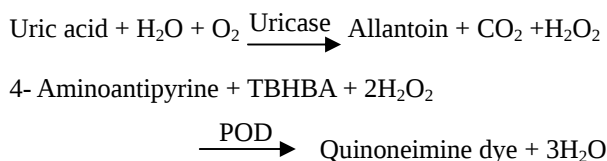
For sample preparation (Urea by Berthelot method – a kit supplied by Aspen Laboratories, Baddi, Himachal Pradesh, India) was used. To 10 μ l of the serum first 50 μ l of urease enzyme was added and incubated for 5 minutes at 37° C. Then 1.5 ml of phenol reagent and 1.5 ml of hypochlorite reagent were added and incubated for 20 minutes at 37° C. The principles involved for this reaction are follows:



Urease hydrolyses urea into ammonia and carbon dioxide. In alkaline conditions, ammonia reacts with hypochlorite and phenol in the presence of nitroprusside to form Indophenol colored complex. The intensity of the color is directly proportional to the concentration of urea in the sample.

2.2. Uric Acid Sample Preparation

1 ml of uricase enzyme was added to 20 μ l of serum (Uricase based on POD - a kit supplied by Merck, Mumbai, India). The solution was mixed well and incubated at 37° C for 10 minutes. The principle involved in this reaction is represented as:



Uric acid is hydrolyzed by Uricase to allantoin and hydrogen peroxide. Hydrogen peroxide so formed reacts with 4-aminoantipyrine and 2, 4, 6-tribromo-3- hydroxy benzoic acid (TBHBA) in the presence of enzyme peroxidase (POD) to produce Quinoneimine dye compound. The intensity of the color is directly proportional to the concentration of uric acid in the sample.

2.3. Nonlinearity Measurements

Z-scan experiments were performed using a 532 nm Nd: YAG (SHG) CW laser beam (COHERENT –Compass 215M-50 diode-pumped laser) focused by a lens of 35 mm focal length. The experimental set up is shown in

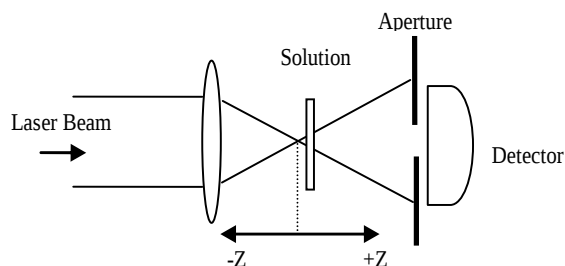


Figure 1. Experimental setup for Z-scan technique.

Figure 1. A typical closed-aperture Z-scan curves for the standard urea and uric acid solution at incident intensity $I_0 = 7.824 \text{ kW/cm}^2$, are shown in **Figure 3**. This normalized transmittance curves are characterized by a pre-focal peak followed by a post-focal valley. This implies that the nonlinear refractive indices of urea, uric acid are negative ($n_2 < 0$). The defocusing effect shown in Z- scan curve can be attributed to a thermal nonlinearity resulting from absorption of radiation at 532 nm. Localized absorption of a tightly focused beam propagating through an absorbing sample medium produces a spatial distribution of temperature in the sample solution and consequently, a spatial variation of the refractive index, that acts as a thermal lens resulting in phase distortion of the propagating beam.

The nonlinear refractive index (n_2) is calculated using the standard relations [18].

$$\Delta T_{p-v} = 0.406(1-S)^{0.25} |\Delta \Phi_0| \quad (1)$$

where ΔT_{p-v} can be defined as the difference between the normalized peak and valley transmittances ($T_p - T_v$), $|\Delta \Phi_0|$ is the on-axis phase shift at the focus.

The linear transmittance of the aperture is given by

$$S = 1 - \exp(-2r_a^2 / w_a^2) \quad (2)$$

where r_a is the radius of the aperture and w_a is the beam radius at the aperture.

$$n_2 \approx \frac{\Delta \Phi_0}{k I_0 L_{\text{eff}}} \quad (3)$$

where n_2 is the nonlinear refractive index, k is the wave number ($k = \frac{2\pi}{\lambda}$) and

$$L_{\text{eff}} = \frac{1 - e^{-\alpha L}}{\alpha}$$

$I_0 = \frac{2P}{\pi w_0^2}$ is defined as the peak intensity within the

sample at the focus. L is the thickness of the sample, α is the linear absorption coefficient.

The results are checked by performing conventional colorimetric method following the standard procedure of

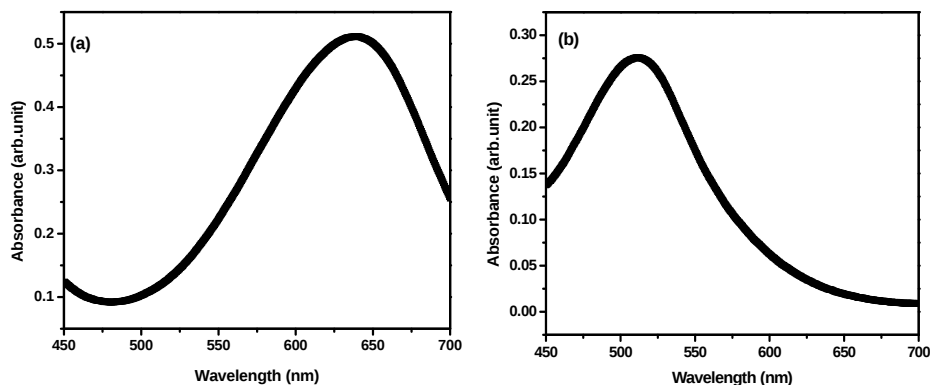


Figure 2. UV-Vis Spectra of standard (a) urea (b) uric acid with reagent.

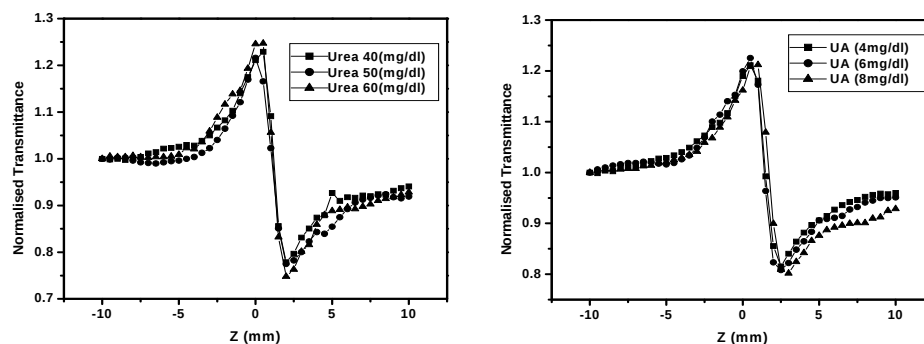


Figure 3. Z-scan data of the standard urea and uric acid (UA).

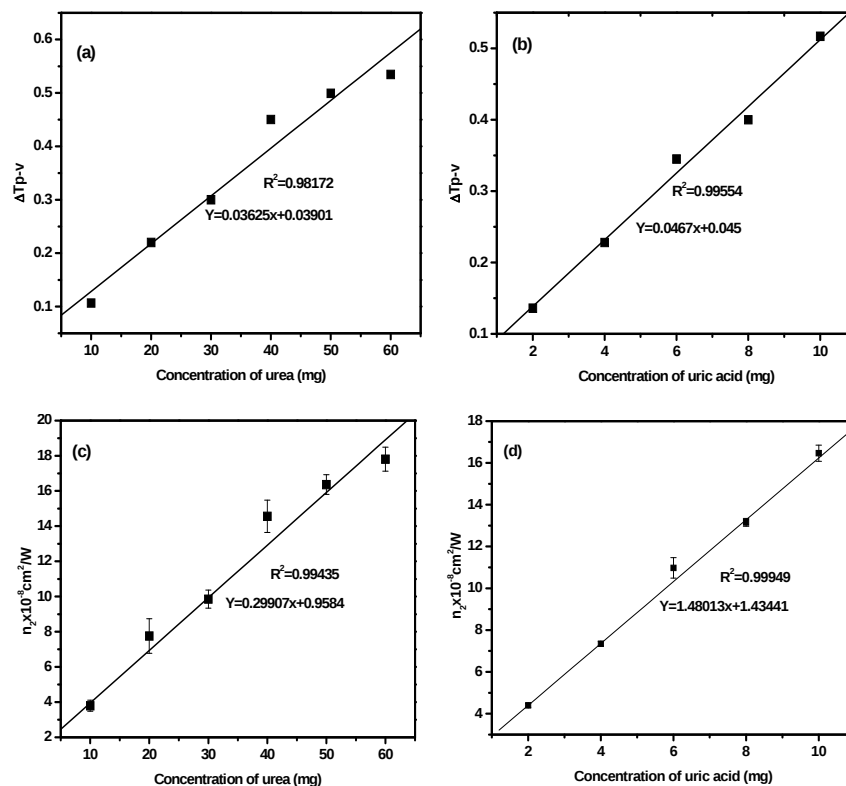


Figure 4. Linear variation of T p-v and nonlinear refractive index (n_2) with concentration of urea (a,c) and uric acid (b,d) by Z-scan method.

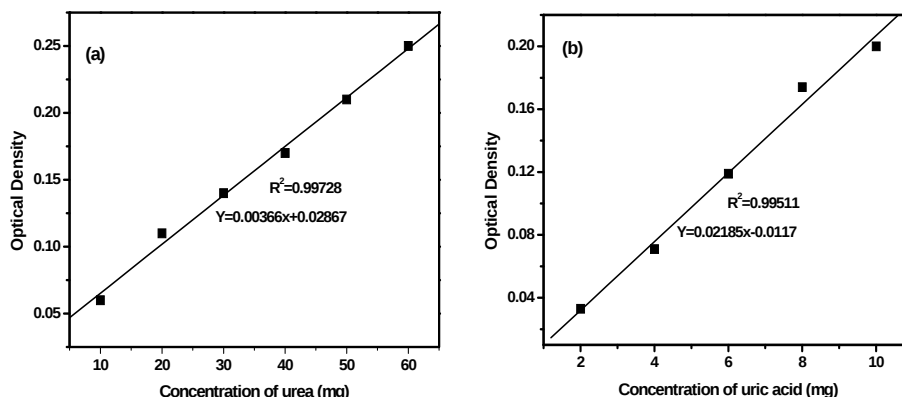


Figure 5. Linear variation of optical density of urea (a) and uric acid (b) by colorimetric method.

A. J. Taylor *et al.* [23] for urea and P. Fossati *et al.* [24] for uric acid. This involves measurement of optical density variation with respect to concentration as shown in Figure 5. The results thus obtained are compared with the results calculated with the Z-scan technique.

2.4. Statistical Analysis

The error involved in the measurements was determined by *t* test, $P < 0.01$. These statistical analysis was conducted using SPSS commercial statistical package (SPSS, version 10.0 for windows, SPSS Inc., Chicago, U.S.A).

3. RESULTS AND DISCUSSION

3.1. Spectral Absorbance Measurements

The absorption spectra were measured using UV-Vis spectrophotometer (SHIMADZU-UV-2401PC), and the spectra for both urea and uric acid were found to be broad banded as depicted in Figure 2. Both have exhibited good absorption at 532 nm. Hence for further study 532 nm Nd: YAG laser was used.

3.2. Nonlinear Refractive Index

The results of typical Z-scan normalized transmittance measurement for urea and uric acid are shown in Figure 3. As the concentration of the standard urea and uric acid increases, the normalized transmittance peak increases whereas the valley decreases respectively.

It is found that ΔT_{p-v} as well as refractive index value increase linearly with concentration of standard urea and uric acid as seen in Figure 4(a) to (d). Figure 5(a) and (b) shows the linear variation of optical density with concentration of urea and uric acid respectively as measured with conventional colorimetric method.

The experiments were repeated five times and the mean value of the nonlinear refractive index (n_2) was calculated from the normalized transmittance values. This calculated value was assumed to be the standard for measurement of unknown urea and uric acid content pre-

sent in blood sample. This was arrived by plotting a linear graph of urea and uric acid concentration Vs non-linear refractive index. The nonlinear refractive index value was first measured against the reagent blank solution.

The calibration was made with the conventional colorimetric method and the results are tabulated in Table 1 for urea and Table 3 for uric acid. The common urea level in blood serum is 10 – 50 mg/dl. The common uric acid level in blood serum is 3.4 – 7.0 mg/dl range for Males and 2.5-6.0 mg/dl range for Females.

To calculate the urea and uric acid levels, one need not

Table 1. Nonlinear refractive index (n_2) values for standard urea.

Standard Urea Concentration (mg/dl)	Nonlinear refractive index $n_2 \times 10^{-8} (\text{cm}^2/\text{W})$
10	03.79 ± 0.31
20	07.75 ± 0.98
30	09.85 ± 0.51
40	14.55 ± 0.91
50	16.36 ± 0.55
60	17.80 ± 0.68

Table 2. Comparative analysis of serum urea measurement using colorimetric method and Z-scan method.

Sample Collection	Urea level	Concentration of urea (mg/dl)	
		Colorimetric method	Z-scan method
Male	Normal	34.74	35.08
Female	Normal	33.82	33.12
Male	Normal	42.82	42.24
Female	Normal	31.46	32.03
Female	Normal	26.64	25.85
Male	Normal	38.08	38.99

Each value is the mean of 5 individual observations. The P value (t-test value) is less than 0.01 at 1% significance level.

Table 3. Nonlinear refractive index (n_2) values for standard uric acid.

Standard Uric acid concentration (mg/dl)	Nonlinear refractive index $n_2 \times 10^{-8} (\text{cm}^2/\text{W})$
2	04.39 ± 0.09
4	07.34 ± 0.10
6	10.97 ± 0.48
8	13.15 ± 0.18
10	16.46 ± 0.38

Table 4. Comparative analysis of serum uric acid measurement using colorimetric method and Z-scan method.

Sample collection	Uric acid level	Concentration of uric acid (mg/dl)	
		Colorimetric method	Z-scan method
Female	Normal	3.40	3.37
Male	Normal	5.55	5.61
Male	Normal	5.47	5.46
Female	Normal	4.05	4.12
Male	Normal	6.28	6.26
Female	Normal	4.35	4.34

Each value is the mean of 5 individual observations. The P value (t-test value) is less than 0.01 at 1% significance level.

plot full Z-scan curve every time. Once, experimental setup explained above is established, one needs to note down peak and valley values of the transmittance curve translating the sample holder continuously along Z- axis.

The difference in these two values $T_p - T_v$, $|\Delta\Phi_0|$ when substituted in Eq.(3) yields the nonlinear refractive index value [17].

From the Z-scan results we infer that, the nonlinear refractive index n_2 values for the common level of urea in blood serum (10-50 mg/dl) are 03.79 ± 0.31 and $16.36 \pm 0.55 \times 10^{-8} \text{ cm}^2/\text{W}$ respectively.

Likewise, for common level of uric acid in blood serum for males 3.4-7.0 mg/dl and their corresponding n_2 values are 6.45 and $11.78 \times 10^{-8} \text{ cm}^2/\text{W}$ respectively. For females 2.5-6.0 mg/dl and their corresponding n_2 values are 5.15 and $10.97 \pm 0.48 \times 10^{-8} \text{ cm}^2/\text{W}$ respectively.

3.3. Evaluation with Conventional Method

Many trials were performed to measure the urea and uric acid level with our proposed method. The blood samples were collected from six volunteers. (Three males and three female). We could see that the results arrived are in good agreement with those of the conventional colorimetric method for urea Table 2 and for uric acid Table 4. This ascertains that the proposed method is on equality with the conventional colorimetric method.

4. CONCLUSIONS

We have measured the nonlinear refractive index values for urea and uric acid present in the serum sample by Z-scan method with 532 nm Nd:YAG CW laser. The Z-scan measurements indicate that urea and uric acid in standard sample and blood sample exhibit nonlinear optical properties. Comparative analysis of these values with the one obtained by conventional colorimetric method shows that they are in good agreement. Thus Z-scan technique is found to be suitable for measurement of bioanalytes.

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Study on promoting quantum mechanics-teaching modernization by information technology

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ABSTRACT

Quantum mechanics, one of the important theories of modern physics, is fairly esoteric and abstract. Based on the characteristics of the quantum mechanics course, the article studies the modernization of quantum mechanics teaching in four aspects: the modernization of the teaching idea, Computer-Aided Instruction, the development of Information technology software and the establishment of three-dimensional digital curriculum teaching resource library.

Keywords: Quantum Mechanics; Information Technology; Modernization of Teaching

1. INTRODUCTION

Information technology is the general appellation of communication technology, computer technology, multimedia technology and network technology. In recent years, information technology has been applied in all walks of life, greatly promoting the social progress and economic development; meanwhile, it has also provided the more vast development space for higher education, thus bringing significant changes to higher education content, form, methods have undergone [1]. Quantum mechanics, as one of the two pillars of modern physics and the most important required course for physics majors, is an important basis for some post-graduate courses such as theoretical physics, materials science, laser physics and technology, bio-physics, and theoretical chemistry. Through the study of this course, students can master the basic conceptions and theories of quantum mechanics and acquire the ability to analyze and solve problems with the basic theories of quantum mechanics. But it is very difficult for the beginners to accept the theory, for it is fairly esoteric, abstract and complex. Modern information technology can integrate and

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construct an ideal learning environment effectively, which can support the creation of the real situation, the sharing of resources without the limit of time and space, the fast and flexible obtaining of information, and the interaction of rich resources, thus leading to new learning methods such as research-based learning and cooperative learning, and achieving the goals of deepening the teaching reform and training innovative talents. So the introduction of information technology in the teaching of quantum mechanics can effectively promote the modern teaching of quantum mechanics.

2. UPDATE THE IDEA OF TEACHING, AND ENHANCE THE UNDERSTANDING OF MODERN TEACHING TECHNIQUES

Education modernization demands not only the renewal of equipment and technology but also of the teaching idea. Updating teaching idea is to change the educational idea, teaching content and teaching methods, which are behind the times. Traditional teaching is based on the basic purpose: teaching students knowledge, telling students the way to live and answering students' questions, and teaching knowledge is the fundamental goal of teaching. This old teaching model and teaching idea do not fit in with the law of education and hinder the improvement of the students' physical quality, thus being not conducive to the cultivation of talents. To cultivate talented personnel of high qualities who meet the new age's needs and intense competition, we must reform education ideology, instruction content and instruction means. Computer is the basis and the core of modern science and technology. Mankind has entered the computer age and the information superhighway era. Moreover, more than 90% of the invention and development of the world science and technology is closely linked with the computer technology in recent 30 years. It can be said that commanding the knowledge of computer is an essential quality for the future talents. As for the requests for the next generation of young people, the em-

phasis should not be put too much on the amount of the knowledge they have reserved, but on their ability to obtain knowledge.

Computer and software are the crystallization of the wisdom and a valuable knowledge product. As an important component of modern teaching technology, Computer is introduced to classroom teaching and family tutoring, which will not only be good for us to strengthen the times, competitiveness and pathbreaking of the teaching and to face the challenges of a new era, but also help students form new ideas, new concepts and new methods in the learning process, which is very important for them to become a high-quality personnel in future.

3. THE MAIN WAY TO PROMOTE THE TEACHING MODERNIZATION BY INFORMATION TECHNOLOGY

3.1. Computer-Aided Teaching Enriches the Class Teaching Method

The enrichment of quantum mechanics teaching content, the decrease of teaching hours and the improvement in the request of talent training quality make the contradictions of the teaching enforcement process more obvious, so it is essential to put forward the best solution to solve the problem to enhance the effectiveness of teaching. There are some obvious shortcomings in traditional class teaching and the lag of teaching technology led to rigid forms of class teaching. The original teaching pattern, in which teachers teach and students listen and take notes, hindered the students' interest in study. Therefore it is necessary to change the writing on blackboard simply into electronic teaching plan, writing on blackboard, video, CAI, demonstration of the experiment and the network to achieve the best teaching results. It is worth mentioning CAI, the character of which is the visualization and diversification of teaching content, the rigorous and scientific nature, the unified applicability and timely interaction, which favors the implementation of individualized teaching. CAI, as an effective means of modern teaching, is getting more and more favor of teaching workers. The emergence of CAI brought a vitality for class teaching, and its powerful demonstration function can not be compared by traditional equipment.

3.2. Digital Software Development for Typical Teaching Content

Quantum effects, quantum model, quantum theory, quantum computing, quantum experiment and quantum application in quantum mechanics are very difficult and abstract to understand, so the teaching effect has been less prominent. But it has been proved by practice that the development of proper software for teaching will

achieve better results.

3.2.1. Make Full Use of the Three-Dimensional Demonstration of Information Technology to Learn Abstract Wave Function

Comparing quantum states with the state statistics in the teaching of wave function, accepting wave function with the idea of distribution function is called the probability density [2]. Then, make a surface of the equivalent probability density, and the probability of emergence of micro-particles is equal. It will be fine to find the dot (r, θ, ϕ) , which satisfies the equation $\psi[r, \theta, \phi] = \text{const}$, and then to run the dot in a smooth surface (Figure 1, Figure 2).

3.2.2. Play a Numerical Calculation and Simulation Capabilities to Achieve the Strengthening of the Basic Concepts of Quantum Theory and the Experiment Content in the Classroom

Movement of micro-particles is random in general. The description of the motion of the wave function is the probability (density) range, and eigenvalue and eigenstate reflects the side of movement determinism. That is, the measured corresponding value of physical quantities is determined when the particle is in a mechanical eigenstate.

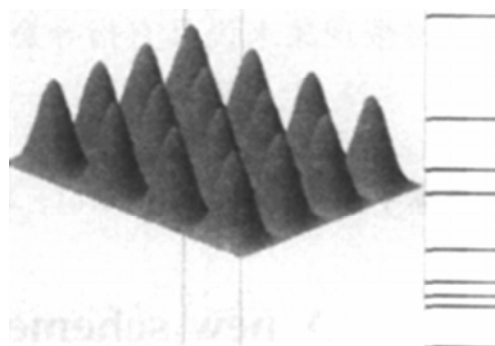


Figure 1. Probability density two-dimensional infinite well wave.

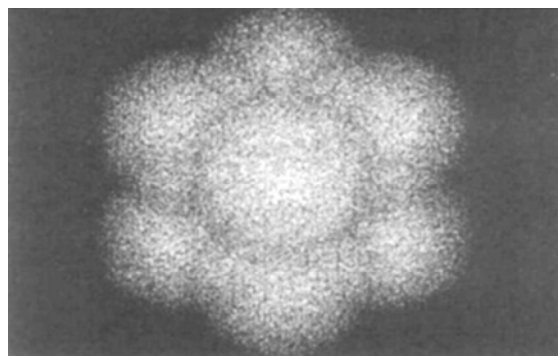


Figure 2. Three-dimensional infinitely deep well the probability density-wave.

The calculation and solving on eigen equation, the eigenstate and eigenvalue of the mechanical quantity is the important teaching content and methods to understand the basis of quantum theory. However, it is difficult to study when facing the energy spectrum of one-dimensional finite deep square potential trap, so we can make a potential trap spectrum distribution of different wide with digital technology (**Figure 3**). We will feel more relaxed by studying the energy spectrum of one-dimensional finite deep square potential trap by diagram.

In addition, when introducing the teaching of quantum experiments [3], we can take full advantage of the digital calculation and simulation technology to show students quantum fence and quantum decoherence, which are difficult to understand, and instruct students to realize the simulation by programming (**Figure 3**, **Figure 4**). The technology is also widely used in the teaching Spatial Orientation quantization of angular momentum, two-dimension brillouin zone of energy band theory and comp-ton scattering experiment with perfect teaching result.

3.2.2. Set Up Three-Dimensional Digital Teaching Resources of Quantum Mechanics

The characteristics of college students learning are the combination of class learning with extra-curricular learning. But extra-curricular learning resources in many schools are the teaching plan of teachers and the answer to exercises, etc, which can not provide effective help to

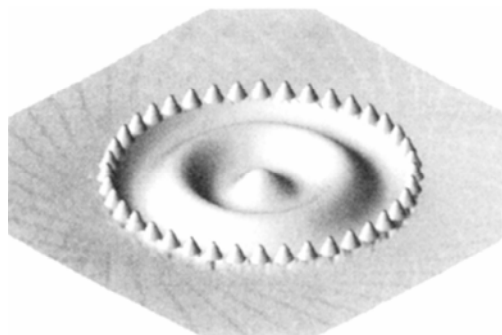


Figure 3. Quantum corral.

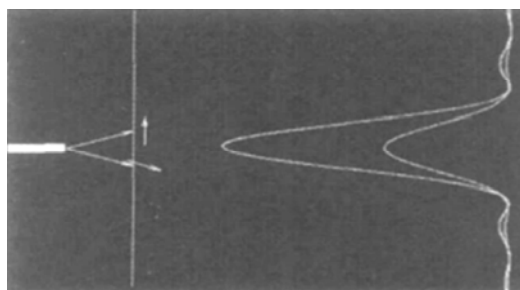


Figure 4. Quantum decoherence.

students. Information technology has been promoting the reform and innovation of the traditional audio-visual and computer-assisted education since the mid 90s of last century. Fine quality learning resources on quantum mechanics can be found in network learning resources at home and abroad at present and the resources are very rich; therefore, we can collect high-quality digital teaching resources about quantum mechanics at home and abroad, and then select, integrate and create the existing digital teaching resources by combining the teaching practice with teaching research of chief teachers in accordance with the new education ideas, with the aid of modern information technology to build three-dimensional digital resource library and at the same time to assist the teaching resources on the Net which include teaching software, management software and Information Consultation. It is especially important to establish the multi-dimensional database of information integrable ware according to the thought of the integrable ware, and teaching on Net becomes true by using the integrable ware to assemble a platform. Provide students with a broad three-dimensional self-learning space for students' extra-curricular learning. (Shandong Province, the provincial Quality Course Digital Web site of quantum mechanics: <http://222.133.5.50/kc/lzlx/index.php>)

4. CONCLUSIONS

Nonesuch Courses Building is one of the important contents of the Project of Teaching Quality and Teaching Reform in Colleges, the aims of which is to improve the teaching quality and the quality of personnel training [4]. Information technology is a powerful backup of the realization of the teaching modernization and a necessary condition of Course Construction. It can be predicted that the further development of information technology will promote quantum mechanics-teaching modernization to a higher level.

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Optimization of clear liquid fermentation condition for ethanol production from *Canna edulis* Kerl

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ABSTRACT

In this paper, clear liquid fermentation condition for ethanol production from *Canna edulis* Kerl with orthogonal design was studied. We have studied five factors that influenced clear liquid fermentation condition for ethanol production from *Canna edulis* Kerl by the single-factor test. The five factors were α -amylase amount, glucoamylase amount, pH, nitrogen and auxiliary materials amount. Selected the best amounts of α -amylase, glucoamylase, pH, nitrogen source and auxiliary materials by means of the single-factors test. And then, we used the Orthogonal test to optimize the fermentation process. The study showed that the best fermentation conditions were α -amylase amount 7 u/g *C. edulis*, glucoamylase amount 145u/g *C. edulis*, pH 4.4 and the amount of urea 0.08%, 1% of rice bran as auxiliary materials. The rate of alcohol which was produced by the best fermentation conditions is 21.5% that increased by 1.3% than the rate of alcohol by the single-factor test.

Keywords: Alcohol; *Canna edulis* Kerl ; Clear Liquid Fermentation; Orthogonal Design

1. INTRODUCTION

Canna edulis Kerl belongs to *Cannaceae* and *Canna* L, in Chinese herbs it was called jiang-yu or jiao-ou. At the first of 20th century, it was planted widely at slope and hills in Sichuan, Guizhou and Yunnan because of its excellent feature. The content of starch in rhizomes of *Canna edulis* Kerl is very abundant. The content of starch is about 40%–60% [8-13]. So *Canna edulis* Kerl has great development potential in supply of starch. The planting of *Canna edulis* Kerl can increase earning for mountain people. At the same time it has continuous development significance in regulating agricultural configuration and developing rural economics. But the starch of *Canna edulis* Kerl is made into noodle

largely only now, so its value is low. The molecular structure and physical and chemical properties of the starch of *Canna edulis* Kerl were studied largely. Now, many scholars are investigating other purposes of the starch of *Canna edulis* Kerl. For example, the starch of *Canna edulis* Kerl has achieved a breakthrough in alcoholic fermentation. The solid-state fermentation, clear liquid fermentation of *Canna edulis* Kerl and the comprehensive utilization of fermentation residue have been researched largely in laboratory [1,7-11]. On this basis, we have got the best clear liquid fermentation process from *Canna edulis* Kerl with the single-factor test. Then we improve the utilization rate of *Canna edulis* Kerl raw after optimizing the techniques of clear liquid fermentation condition for ethanol production from *Canna edulis* Kerl [8,12,15,16].

2. MATERIALS AND METHODS

2.1. Experimental Material

Canna edulis Kerl: Collected from Xingyi Guizhou, the content of Starch is 60.1%, the content of moisture is 14.8%;

Thermal Resistance α -amylase: wuxi xing-da Bio-Engineering Co., Ltd., enzyme activity: 20000u/mL;

New liquid glucoamylase: wuxi xing-da Bio-Engineering Co., Ltd., enzyme activity 100000u/ML.

2.2. Methods

2.2.1. The Preparation of Clear Liquid Culture Medium

Firstly, mixed the powder of *Canna edulis* Kerl with water in a certain proportion, joined in α -amylase, then heated them to 85~90°C rapidly, keep them together 30~60min. When the time was over, cooled them to 60°C, adjusted pH 4.2. A certain proportion moderate glucoamylase was joined into the mixture solution, a few minutes later, the Solid-state glycation Saccharification dregs and clear liquid can be gained by centrifugal technology, then put the clear liquid into the autoclave whose

temperature was 121°C, the clear liquid was taken out after twenty minutes. When the clear liquid temperature fell to 30°C, the yeast which was activated was inoculated into the clear liquid, the fermentation started in the incubator [2,3].

2.2.2. Determination of Alcohol Volume Fraction

The clear liquid, which was the outcome of *Canna edulis* Kerl fermented, was measured 100mL by measuring graduates (100mL), inoculated into a rockered flask (500mL) which contained 100mL distilled water. The distillation was collected by volumetric flask (100mL), determined alcohol level by alcohol meter [4-6].

2.2.3. Calculation of the Producing Ethanol Rate of Raw Materials and Starch Utilization [17]

The producing ethanol rate of raw materials =

$$\frac{95\% \text{ ethanol yield (kg)}}{\text{amount of raw materials (kg)}} \times 100\%$$

The starch utilization =

$$\frac{\text{the actual liquor rate of starch}}{\text{the theoretical liquor rate of starch}} \times 100\%$$

2.2.4. Determination of Reducing Sugar Concentration

The distilled water was inoculated into the volumetric flask which contained separated clear liquid 0.1mL till 25mL. The mixed solution was determined for reducing sugar concentration by DNS analytical method [5].

2.2.5. Determination of Mass Concentration of Total Sugar

The distilled water was inoculated into the volumetric flask which contained separated clear liquid 0.1mL till 25mL. The mixed solution was determined for total sugar concentration by H₂SO₄-Pheul analytical method [5].

3. RESULTS AND ANALYSIS

3.1. Single-Factor Test and Medium Optimization

Based on former experiments, we have found the best solid-state fermentation conditions of the ethanol production from *Canna edulis* Kerl, the best conditions were ratio of material to water 1:2.2, α -amylase amount 5u/g C.edulis, liquefaction time 45 min, glucoamylase amount 150u/g C.edulis, saccharification time 23.2 min, pH 5.5, urea 0.1%, fermentation temperature 27.9 °C, ADY 0.15%, fermentation time 48h and inert carrier 1%.

On this basis, the paper studied the clear liquid fermentation conditions of the ethanol production from

Canna edulis Kerl. By a large number of experiments, we found that α -amylase amount, glucoamylase amount, pH and urea amount have an important influence on the whole fermentation technology.

So we were proceeding to the in-depth study for these four factors, these studies as follows:

3.1.1. The Effect of α -amylase Amount on the Alcohol Fermentation

In order to confirm the appropriate amount of α -amylase on clear liquid fermentation from *Canna edulis* Kerl, different amount of α -amylase were tested on the ethanol rate of raw materials (*Canna edulis* Kerl) [7-11,14]. The results were showed in **Figure 1**.

The **Figure 1** showed that when the α -amylase amount was 6u/g C.edulis, the ethanol rate of raw materials (*Canna edulis* Kerl) was highest. So the conclusion was α -amylase amount 6u/g was the best condition on clear liquid fermentation for ethanol production from *Canna edulis* Kerl.

3.1.2. The Effect of Glucoamylase Amount on the Alcohol Fermentation

Glucoamylase played an important role in the clear liquid fermentation for ethanol production from *Canna edulis* Kerl, it can influence the cost of production. Different glucoamylase amount were tested on the ethanol rate of raw materials [7-11].

The different results were revealed in **Figure 2**.

The **Figure 2** described that when the glucoamylase amount exceeded 150u/g, the ethanol rate of raw materials (*Canna edulis* Kerl) was steady. In order to cut down the consumption of glucoamylase, the glucoamylase amount 150u/g was appropriate on clear liquid fermentation for ethanol production from *Canna edulis* Kerl.

3.1.3. The Effect of Different pH Conditions on Alcohol Fermentation

The pH of Clear Liquid fermented were adjusted to 3.6, 4.2, 4.5, 5.0 and 6.0 (the pH of *Canna edulis* Kerl Glycosylated liquid was original) [7-11]. The outcomes were dr-

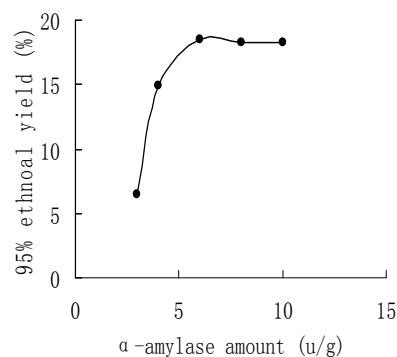


Figure 1. The effect of α -amylase amount on the alcohol fermentation.

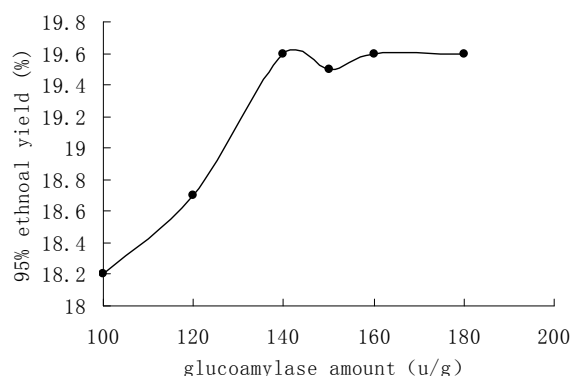


Figure 2. The effect of glucoamylase amount on the alcohol fermentation.

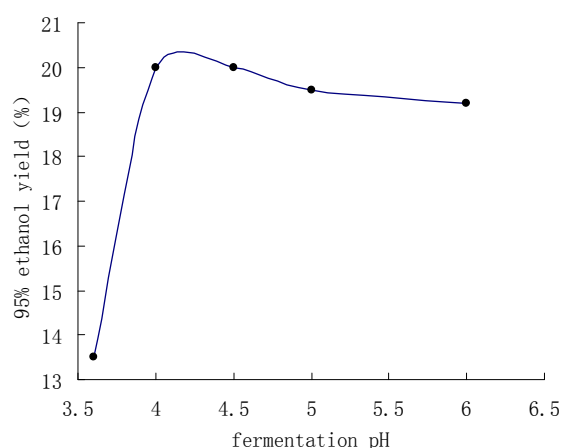


Figure 3. Effect of pH on ethanol fermentation.

ew in Figure 3.

The conclusion can be ascertained: the yield of alcohol was gradual increasing when pH was 3.5 to 4.2. When pH was 4.2 to 6.0, the yield of alcohol had a basically stable situation and the best effect was pH 4.2.

3.1.4. The Effect of Nitrogen on the Alcohol Fermentation

Under suitable environment and nutritional conditions, yeast can bear alcohol in high concentration, but the vigour of yeast was lower when nitrogen was scarce in culture medium. In this article, the yeast was restrained because the amount of nitrogen was deficient in glycosylated liquid. For accelerating growth of yeast and enhancing the ability of alcohol-resistant yeast, a certain amount of nitrogen was necessary to be added in the culture medium. With an eye to the results were various because of different nitrogen, therefore, urea, ammonium sulfate, ammonium bicarbonate, peptone and yeast extract were choiced as the element inspected in this experiment. The amount of nitrogen must be based on 0.1% nitrogen content of urea, the nitrogen content of urea, ammonium sulfate, ammonium bicarbonate, pep-

tone and yeast extract were 46.7%, 20%, 17.7%, 13.5%, 7% respectively. The outcomes were shown in Figure 4.

It was found that five kinds of nitrogen had great influence on alcohol fermentation from *Canna edulis Kerl* although urea showed the best consequence, maybe it can protect some enzymes, for example the alcohol dehydrogenase, promoted the activity of enzyme.

In order to confirmed the best amount of urea, at the next experiment, different dosage of urea which were 0.05%, 0.1%, 0.15%, 0.2% and 0.3% were studied on clear liquid fermentation condition for ethanol production from *Canna edulis Kerl* at the same condition. The results were revealed in Figure 5.

It was showed that the rate of alcohol reduced gradually from 0.15% to 0.3% dosage of urea, the rate of alcohol rised steadily from 0 to 0.1% dosage of urea. The best data appeared at 0.1% dosage of urea.

3.1.5. The Effect of Inert Carrier on Alcohol Fermentation

Fine-Grained material maybe reduce the content of CO₂ which could suppress yeast colony, different content of bran and corn cob were tested respectively on ethanol

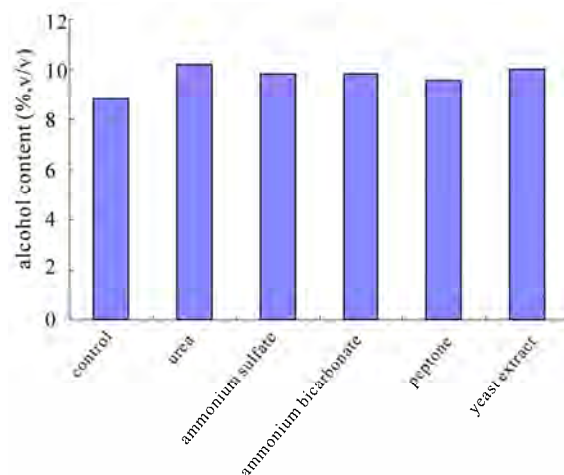


Figure 4. The effect of nitrogen on alcohol fermentation.

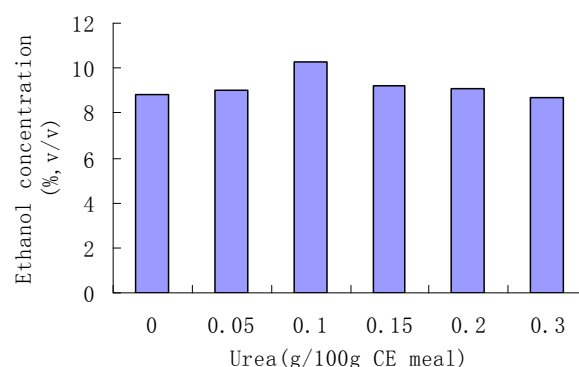


Figure 5. Effect of urea concentration on ethanol fermentation.

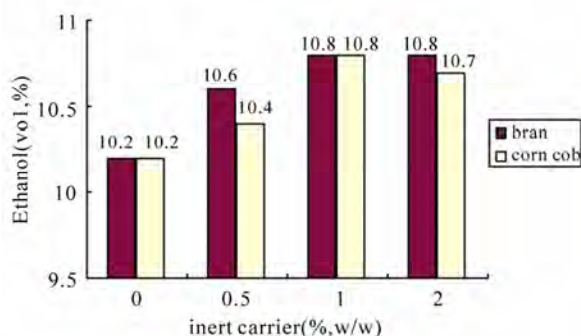


Figure 6. Effect of inert carrier on ethanol fermentation.

fermentation. The content of them were 0, 0.5%, 1%, 2% respectively. The results were showed in **Figure 6**.

The diagram indicated that bran and corn cob can promote alcohol fermentation of *Canna edulis Kerl*. 1% bran had the best effect. The same information had been reported in other documents.

3.2. Orthogonal Test

According to the results of the single-factor test, α -amylase amount, glucoamylase amount, pH and the amount of urea were optimized by orthogonal experiment [8,12,15].

The α -amylase amount 6u/g *C.edulis*, glucoamylase amount 150u/g *C.edulis*, pH 4.2 and the amount of urea 0.1% were used as intermediate level in the orthogonal tests. The experimental design and results of analysis were showed in **Table 1**, **Table 2** and **Figure 7** (In the **Table 1** and **Table 2**, A, B, C and D stand for the

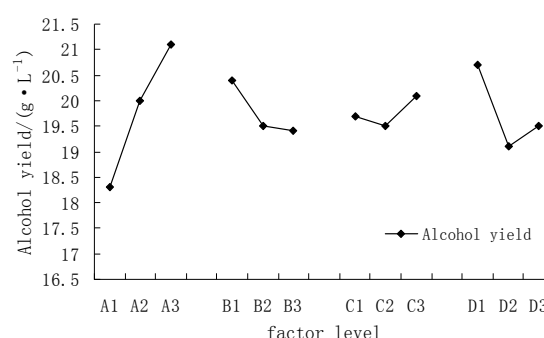


Figure 7. The trend of factors and indexes in orthogonal experiment.

α -amylase amount, glucoamylase amount, pH and urea amount respectively. In the **Tables 1** and **2** stand for three different amount levels of the four factors respectively.)

The **Table 2** showed that α -amylase amount had a great influence on clear liquid alcohol fermentation of *Canna edulis Kerl*. Based on the results of experiment, the influence of the other factors on the clear liquid alcohol fermentation of *Canna edulis Kerl* were ordered from larger to little as the amount of urea>glucoamylase amount>pH.

As can be seen from **Figure 7**, the best combination was $A_3B_1C_3D_1$, that is to say, the α -amylase amount 7u/g *C.edulis*, the glucoamylase amount 145u/g *C.edulis*, pH 4.4, and the amount of urea 0.08%.

At last, after three parallel experiments which have been carried out on ethanol fermentation by the optimal

Table 1. Factor and level of orthogonal experiment.

level	α -amylase amount (u/g)	glucoamylase amount (u/g)	pH	the amount of urea(%)
1	5	145	4.0	0.08
2	6	150	4.2	0.1
3	7	155	4.4	0.12

Table 2. Results and analysis of orthogonal experiment.

Test No.	factor				Alcohol yield(%)
	A	B	C	D	
1	1	1	1	1	19.8
2	1	2	2	2	17.1
3	1	3	3	3	18.0
4	2	1	2	3	20.1
5	2	2	3	1	2.0
6	2	3	1	2	18.8
7	3	1	3	2	21.3
8	3	2	1	3	20.5
9	3	3	2	1	21.4
mean 1	18.3	20.4	19.7	20.7	
mean 2	20.0	19.5	19.5	19.1	
mean 3	21.1	19.4	20.1	19.5	
pole difference	2.8	1.0	0.6	1.7	

technological conditions, we found the production rate of alcohol were 21.7%, 21.4%, 21.5% respectively, and the average was 21.5%.

4. CONCLUSIONS

1) *Canna edulis Kerl* is a non-alimentary crop. The advantages of fuel ethanol produced by *Canna edulis Kerl* were raising the utilization rate of resources, reducing the pollution of the environment, lower manufacturing cost and higher productivity. The fuel ethanol produced from *Canna edulis Kerl* can not only solve the problem of food security, but also promote the development of fuel alcohol industry in China.

2) We have known that α -amylase amount, glucoamylase amount, pH, nitrogen and auxiliary materials can influence clear liquid fermentation for ethanol production from *Canna edulis Kerl*. Then optimizing four factors by the orthogonal test. The best process conditions are α -amylase amount 7u/g *C. edulis*, glucoamylase amount 145u/g *C. edulis*, pH 4.4, the amount of urea 0.08% and 1% of rice bran. The production rate of alcohol was 21.5% which increased by 1.3% than the production rate of alcohol by the single-factor test.

3) We are researching the best conditions of clear liquid fermentation for ethanol production from *Canna edulis Kerl*. These investigations laid a good foundation for reducing the cost of alcohol production, comprehensive utilization of Resources and efficient, saving, undeveloped techniques of alcohol production. So clear liquid fermentation for ethanol production from *Canna edulis Kerl* was an epoch-making event in the realm of industrial alcohol.

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A new method of building permeability model in low-permeability reservoir numerical simulation

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ABSTRACT

Aiming at solving the problem that big difference exists between logging permeability and true permeability of micro-fractured low-permeability sand reservoir, this paper puts forward a new method to revise logging permeability by using primiparity data of oil field. This method has been successfully applied to revise logging permeability of micro-fractured low-permeability sand reservoir in Baiyushan area of Jing'An oil field, which shows that the method is reliable because the geological model building through the permeability which has been handled by this method accords with the real reservoir significantly.

Keywords: Logging Permeability; Micro-Fractured; Low-Permeability Reservoir; Revise

1. INTRODUCTION

In recent years, about 60 to 70 percent of discovered reserves are low-permeability ones, it's anticipated that most of discovered reserves in the future will be low-permeability reservoir, how to develop it efficiently is getting counted [1-3]. Development plan and effect are determined by the performance characteristic research of low permeability reservoir, permeability is a key factor characterizing fluid flow. There are lots of problems in the evaluation of permeability in micro-fractured low-permeability reservoir. The relationship between logging and core analysis permeability, which is caused by developed fracture, is too complicated to be used in reservoir engineering and numerical simulation [4-5]. Due to non-linear fluid flow, the actual flow characteristics of reservoir couldn't be reflected by logging permeability and core permeability correctly. For solving reservoir performance evaluation of oil field development production, the performance permeability used in research, the actual flow characteristics of reservoir could be reflected

by which of test and primiparity data, is very important for logging permeability corrected method.

2. AIBRATION CLCULATION POCEDURE OF LGGING PRMEABILITY

2.1. Calculating Reservoir Permeability Using Primiparity Data Files

Since particularity of micro-fracture in low-permeability reservoir, if logging permeability is used for reservoir engineering evaluation and numerical simulation calculation directly, severe deviation will occur, causing the inaccuracy of result. However, reservoir development performance is the objective behavior of reservoir actual flow characteristic. Performance permeability, calculated by reservoir development performance files, is the true permeability in reservoir. During oil field production, well yield [4] is usually calculated by the equation of radius fluid flow deliverability. On the contrary, reservoir performance permeability is solved by that equation, concrete Equation is written below [6-10]:

$$K_T = \frac{Q \cdot \mu \cdot \ln \frac{r_e}{r_w}}{2\pi \cdot h \cdot \Delta P} \quad (1)$$

where K_T is reservoir dynamic permeability, μm^2 ; Q is well yield, cm^3/s (sub); μ is oil viscosity in formation, mPa s ; r_e is supply radius, cm ; r_w is oil well radius, cm ; h is reservoir thickness, cm .

For the convenience of calculation, standard units of Darcy law are used in the variables above. When practically applied, well yield needs to be conversed to sub-surface volume with volume factor. Half of well spacing could be substituted for r_e , because r_e is in the range of logarithm, with small effect on the results.

2.2. The Calculation of Reservoir Logging Permeability

The result of sequent point by point treatment in log interpretation model is usually used for logging perme-

ability, since the interval of log sample density is 0.125m, and performance permeability calculated by actual production files is located in some production interval, there is asymmetry between them. Logging permeability in target stratum must be averaged by actual production intervals, in order to build corresponding relationship with the permeability of actual production intervals. The permeability expression of actual production intervals calculated by average method of logging permeability is described below:

$$\bar{K}_L = \frac{\sum_{i=1}^{n_2} \left[\frac{\sum_{j=1}^{n_1} K(\phi, Mz, V_{sh})_j}{n_1} \right] \cdot h_i}{h} \quad (2)$$

where $\bar{K}_L$ is the average logging permeability of reservoir production intervals, μm^2 ; $K(\phi, Mz, V_{sh})_j$ is the permeability of j sample site in i sublayer, μm^2 ; ϕ , Mz , V_{sh} is the porosity, the median grain diameter, the shale content of j sample site in i sublayer; h_i is the thickness of reservoir production intervals, m; n_1 is the number of logging permeability point in i sublayer; n_2 is the number of sublayer in piezometric intervals.

2.3. The Correction of Logging Permeability

Reservoir performance permeability, calculated by primiparity data files, could reflect the actual flow characteristics in reservoir, which is closer to the true one. Because there is great distinction between logging permeability and performance permeability in fractural low-permeability reservoir, when logging permeability is corrected by performance permeability, the correction coefficient λ has to be introduced, the λ can be calculated by **Formula 3**:

$$\lambda = \frac{K_T}{\bar{K}_L} = \frac{\frac{Q \cdot \mu \cdot \ln \frac{r_e}{r_w}}{2\pi \cdot h \cdot \Delta P}}{\sum_{i=1}^{n_2} \left[\frac{\sum_{j=1}^{n_1} K(\phi, Mz, V_{sh})_j}{n_1} \right] \cdot h_i}{h} \quad (3)$$

where λ is correction coefficient which is revise logging permeability to performance permeability, the values varies by wells. The greater of λ , the more difference between logging permeability and performance permeability, the logging permeability can't reflect reservoir effluent seepage characteristic. The less of λ , the less difference between logging permeability and per-

formance permeability, the logging permeability can approximately reflect reservoir effluent seepage characteristic.

As to every production well, only one λ value can be calculated by **Formula 3**, but, when building 3D permeability model, the every point logging permeability need to be revised. Only using every point revised permeability, the 3D permeability model can better reflect reservoir actually effluent seepage characteristic. So the **Formula 4**, dynamic correction model of logging permeability, can be used to correction every point logging permeability.

$$Perm = \lambda \times K(\phi, Mz, V_{sh}) \quad (4)$$

where $Perm$ is logging permeability after performance correction, for reservoir permeability model, μm^2 ;

With the value of λ , logging permeability of each well could be corrected to performance permeability point by point, then performance permeability model, which is closer to the true reservoir, would be established for the research of reservoir engineering and numerical simulation.

When using this method, the problems below should be noticed. 1) In order to ensure that only single phase flow occurs, initial production should be taken as possible; 2) Well flow rate must be conversed to sand face one by volume factor; 3) Don't use multiple zones commingled test data; 4) If the area of reservoir is large, fluid viscosity varies, it needs to be treated zone by zone.

3. APPLICATION

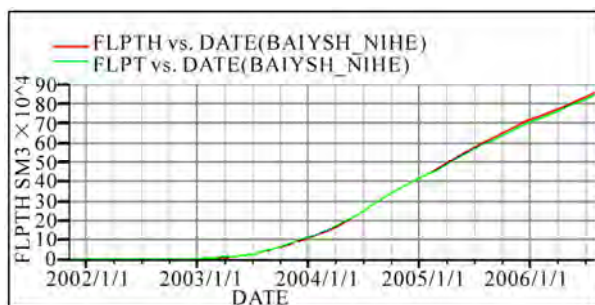
In this paper, Chang 4+5 reservoir in Baiyushan area of Jing'An oil field is chosen as an example. Baiyushan area of Jing'An oil field is situated at NE-SW nose like structure on the Shanbei slope of Ordos basin, Chang 4+5 reservoir is generally delta front subfacies deposit, major reservoir is subsea distributary channel sand, most of reservoir lithology are fine sandstone, siltstone and argillaceous siltstone; Major reservoir sand type is lithic arkose; The average porosity of reservoir is 12.4%, the average permeability of reservoir is $1.05 \times 10^{-3} \mu\text{m}^2$, it's a typical sand reservoir with low porosity and permeability. Structural microfracture developed in reservoir has been discovered during production. When production history is matched with the model established by logging permeability, no matter how to regulate relative permeability, it's shown that match water injection is smaller than true one, match oil production is smaller than true one.

According to logging permeability correction method above, logging permeability performance correction coefficient λ of wells in region of interest were calculated (**Table 1**, partial wells shown). From **Table 1**: it's seen that great difference exists between performance

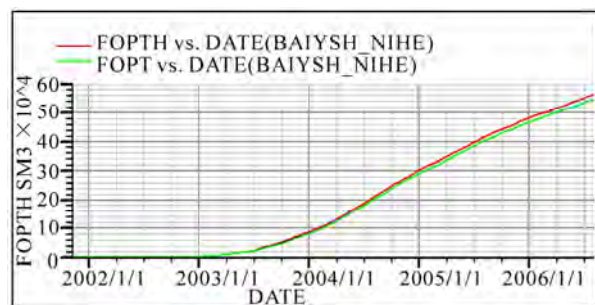
Table 1. Log interpretation permeability dynamic correction coefficient.

Well name	Daily rate (m ³ /d)	Drawdown (MPa)	Thickness (m)	Logging perme- ability ($\times 10^{-3} \mu\text{m}^2$)	Performance permeability ($\times 10^{-3} \mu\text{m}^2$)	Permeability correction coeffi- cient
Yu35-23	5.78	6.93	7.90	1.03	5.67	5.52
Yu36-22	6.82	6.83	8.60	1.65	6.23	3.79
Yu36-23	7.71	6.83	9.05	1.22	6.69	5.50
Yu37-21	9.93	5.33	8.65	0.76	11.56	15.12
Yu37-23	14.48	7.53	10.50	0.72	9.83	13.65
Yu37-27	10.13	7.53	14.00	0.90	5.16	5.72
Yu38-21	7.28	5.53	8.40	0.84	8.41	10.02
Yu38-22	7.21	5.93	15.60	0.78	4.18	5.34
Yu38-23	12.83	6.73	27.00	0.85	3.79	4.45
Yu38-24	12.94	6.73	3.90	1.25	26.45	21.11
Yu38-30	10.90	6.73	17.70	1.35	4.91	3.65
Yu38-31	9.06	7.23	17.80	1.33	3.78	2.83
Yu38-35	8.69	6.33	14.75	0.51	4.99	9.71
Yu39-25	13.35	7.53	18.45	1.15	5.16	4.47
Yu39-27	23.00	7.53	9.70	0.73	16.90	23.24
Yu39-29	8.76	6.93	9.05	1.00	7.49	7.49
Yu39-33	10.59	6.83	15.30	1.16	5.44	4.68
Yu39-37	8.96	6.33	8.50	1.12	8.94	7.97
Yu40-20	11.71	5.83	11.90	0.62	9.06	14.58
Yu40-21	10.13	5.93	20.10	0.88	4.56	5.19
Yu44-22	14.13	3.73	9.95	0.65	20.43	31.56

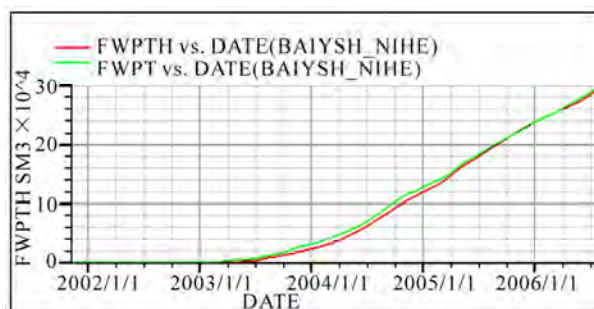
permeability and logging permeability. The biggest was 31.56 times, the smallest was 2.83 times, 5 to 10 times is in general. It was clarified that logging permeability was much smaller than performance one, true flow characteristics of reservoir couldn't be reflected. On the other



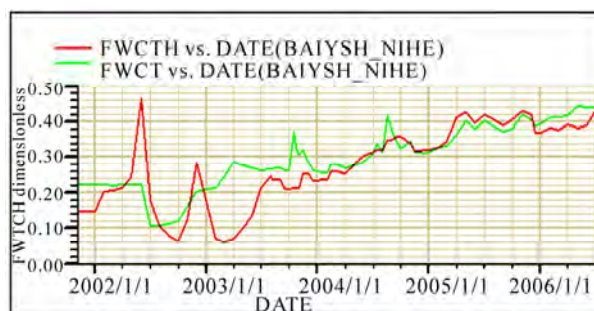
FLPTH: field liquid production total history, FLPT: field liquid production total (model)
(a) Cumulative liquid production



FOPTH: field oil production total history, FOPT: field oil production total (model)
(b) Cumulative oil production



FWPTH: field water production total history, FWPT: field water production total (model)
(c) Cumulative water production



FWCTH: field water cut total history, FWCT: field water cut total (model)
(d) Composite water cut

Figure 1. Development history marching curve of Baiyushan Chang4+5 reservoir in Jing'an oil field.

hand, it was the reason why the actual development of reservoir couldn't be matched using permeability model established by logging permeability directly.

According to the logging permeability correction coefficient of each well, the logging permeability of each well were corrected point by point (0.125p/m), then the dynamic corrected logging permeability of each well were yielded. On basis of it, permeability model of reservoir numerical simulation was established, and producing history was matched. Chart 1 is the result of history match. From the chart 1, we can conclude that, the field liquid production total history is $86.00 \times 10^4 \text{ m}^3$, history match is $84.98 \times 10^4 \text{ m}^3$, error is 1.19% (**Figure 1(a)**); field oil production total history is $49.00 \times 10^4 \text{ m}^3$, history match is $47.65 \times 10^4 \text{ m}^3$, error is 2.76% (**Figure 1(b)**); field water production total history is $29.44 \times 10^4 \text{ m}^3$, history match is $29.98 \times 10^4 \text{ m}^3$, error is 1.84% (**Figure 1(c)**); field water cut total history is 34.2%, history match is 35.3%, error is 3.22% (**Figure 1(d)**); moreover, all the producing history procedure match better (**Figure 1**), the reservoir numerical simulation accuracy requirement is met. It was shown that the method was more coincide with oil field production, better effect of matching.

4. CONCLUSIONS

1) Aiming at the evaluation problem of log interpretation permeability in micro-fractured low permeability reservoir, the correction method of logging permeability using oil field development performance files was proposed, then numerical simulation model, which is closer to the actual permeability in reservoir, was established. The method had a good effect on Baiyushan area of Jing'An oil field.

2) Reservoir numerical simulation model, established by development performance files, is favour of the improvement of reservoir numerical simulation reliability.

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ALICE as a tool for programming at schools

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ABSTRACT

We present some possible application of ALICE in the context of a possible attraction of pupils (especially girls) in early programming course. Our examples presented in the paper are fully explained on the base of [1].

Keywords: Interactive 3D Graphics;
Animation Authoring Tools

1. INTRODUCTION

Alice is an innovative 3D programming environment that makes it easy to create an animation for telling a story, playing an interactive game, or a video to share on the web. Alice is a teaching tool for introductory computing. It uses 3D graphics and a drag-and-drop interface to facilitate a more engaging, less frustrating first programming experience. In Alice's interactive interface, students drag and drop graphic tiles to create a program, where the instructions correspond to standard statements in a production oriented programming language, such as Java, C++, and C#.

This information was found at www site: www.alice.org.

A free ALICE 2.2 programme may be found there, together with some other introductory materials.

Alice allows students to immediately see how their animation programs run, enabling them to easily understand the relationship between the programming statements and the behaviour of objects in their animation. By manipulating the objects in their virtual world, students gain experience with all the programming constructs typically taught in an introductory programming course.

Our aim is to point out that in our opinion this programme may be very useful as a tool for teaching in the Middle schools to attract pupils (especially girls) in: early programming, making short videos with MP3 music and adding some pieces of translations of that music, for example. Other possible applications at school is useful for learning foreign languages, especially English

or German and other subjects as well. We intend to explain our ideas on three examples. These examples were examined on a group of pupils with a positive result.

It is not the purpose of this paper to publish statistical results on an application of the methods proposed but to show how a short presentation at school can be made in order to attract pupils in programming simple videos with their favourite MP3 music or with their own recordings of poems in their own or foreign language. In the third we use special effects. It is obvious that these three simple methods combined together presents a possibility of early introductory into programming in the Middle schools.

2. AN EXAMPLE OF PRESENTING MP3 MUSIC WITH A DOMESTIC OR FOREIGN TEXT

The author of [4] mentioned that: "Women are currently underrepresented in Computer Science". In the opinion of the author of [3] and many others: "middle school is a critical age, during which many girls turn away from scientific and mathematical pursuits, including Computer Science. By giving middle school girls a positive first programming experience, we may be able to increase girl's participation in Computer Science".

Also the opinion above has been made in USA, we have observed that the situation is quite similar in Poland and some other EU countries. In middle schools girls are less interested in Computer science as boys. As the result we observe that girls constitute a minority on Technical Faculties in our Technical Universities (including Opole University of Technology), on Informatics studies, while mathematical studies in Poland, surprisingly, are sometimes occupied by girls (for example at the University of Opole). Despite that fact, we have observed many times, that the scientific way for women, very often, is much more complicated and difficult as for men. However, this is not a topic of that article. Our goal is to concentrate on the particular problem of a possibility of increasing an interest of pupils in Computer Sciences in Middle schools, using the particular programme, namely ALICE. To achieve this goal, we present three possible ways of:

- 1) implementing MP3 popular songs using ALICE,
- 2) implementing poems via ALICE,
- 3) using special effects and sounds.

We have observed that, programming short 1-3 minutes videos with MP3 music is very interesting for pupils, because one may learn programming and train its imagination at the same time, learn the text of the song plus English (or other language) by the way. Therefore we present here an example of a presentation of a piece of one popular song in Germany.

Our translation is a sample of possible texts which may appear in such video. First we present some pictures from the video then a possible code of such presentation, made with ALICE programme.

Example 1.

We present a photo made from a scene of our video: Party.mv by ALICE:



Diagram 1. A photo from video Party.mv.

Party's Code made for a 3 minutes video with MP3 music in German.

Party's Code

Created by: Ewa Graczyńska

World
Events

When the world starts

Do: **world.music**

When A is typed

Do: **ground.dance1**

Let move Any Object

When 1 is typed

Do: **world.dialog1**

When 2 is typed

Do: **girl.dialog2**

Methods

world.music()

No variables

palmTree play sound world.10-AudioTrack 10 (3:44,896)
duration = 150 seconds

world.dialog1 ()

No variables

evilStepsister1 say It's party time! duration = 2 seconds

world.dialog3 ()

No variables

randomGuy2 say You are super! duration = 2 seconds

Ground

Meyhods

ground.dance1 ()

No variables

Do in order

light resize 2 duration = 2 seconds

evilStepsister1 move right 5 meters duration = 1 second

evilStepsister1 turn left 2 revolutions duration = 1 second

Do together

evilStepSister2 turn right 2 revolutions duration = 1 second

palmTree.leaf2 point at evilStepSister2 duration = 2 seconds

evilStepSister2 move amount = 0,25 meters toward target = camera duration = 1 second

Do together

palmTree.leaf6 point at evilStepSister2

evilStepsister1 move left 1 meter duration = 2 seconds

evilStepSister2 move left 1 meter duration = 2 seconds

maleBalletDancer move left 5 meters duration = 2 seconds

Do together

Loop 10 times times

Do together

maleBalletDancer turn left 2 revolutions duration = 2 seconds

Do in order

maleBalletDancer.torso turn at speed left speed = 1 revolutions per second

maleBalletDancer.torso turn at speed right speed = 1 revolutions per second

evilStepSister2 turn left 2 revolutions duration = 2 seconds

evilStepSister2 turn left 2 revolutions duration = 2 seconds

evilStepSister2.stomach.torso roll at speed left speed = 2 revolutions per second duration = 1 second

evilStepSister2.stomach.skirt turn left 2 revolutions duration = 2 seconds

evilStepSister2.stomach.leftThigh turn right 2 revolutions

evilStepSister2 turn left 2 revolutions duration = 2 seconds
evilStepSister2.stomach.rightThigh turn left 2 revolutions
evilStepSister2.stomach.skirt roll at speed left speed = 2 revolutions per second
randomGuy2 point at girl duration = 2 seconds style = abruptly
Do together
maleBalletDancer turn left 2 revolutions duration = 2 seconds
Do in order
maleBalletDancer.torso turn at speed left speed = 1 revolutions per second
maleBalletDancer.torso turn at speed right speed = 1 revolutions per second
evilStepSister2 turn left 2 revolutions duration = 2 seconds
evilStepSister2 turn left 2 revolutions duration = 2 seconds
evilStepSister2.stomach.torso roll at speed left speed = 2 revolutions per second duration = 1 second
evilStepSister2.stomach.leftThigh turn right 2 revolutions
evilStepSister2 turn left 2 revolutions duration = 2 seconds
evilStepSister2.stomach.rightThigh turn left 2 revolutions
evilStepSister2.stomach.skirt roll at speed left speed = 2 revolutions per second
randomGuy2 point at girl duration = 2 seconds style = abruptly
Loop 10 times times
Do together
camera move backward 1 meter duration = 2 seconds
girl stand up duration = 1 second style = gently
girl turn left 0,25 revolutions duration = 2 seconds
girl move left 1 meter duration = 2 seconds
girl turn right 2 revolutions duration = 1 second
girl move forward 1 meter duration = 2 seconds
girl turn at speed left speed = 2 revolutions per second
evilStepsister1.hips orient to palmTree duration = 2 seconds
Do together
girl.body.leftArm turn left 2 revolutions
girl.body.rightArm turn right 2 revolutions
Do in order
girl.body.neck turn left 0,25 revolutions
girl.body.neck turn right 0,5 revolutions
girl.body.neck turn left 0,25 revolutions
girl.leftLeg.lowerLeg orient to

girl.body.leftArm
evilStepsister1.hips.skirt turn to face girl.body
evilStepsister1.hips.leftThigh.leftCalf turn left 2 revolutions
evilStepsister1.hips.rightThigh.rightCalf turn right 2 revolutions
Loop 2 times times
light turn left 1 revolution duration = 2 seconds style = gently
camera get a good look at randomGuy2 asSeenBy = evilStepsister1
randomGuy2 orient to camera duration = 2 seconds
randomGuy2 turn right 0,5 revolutions duration = 2 seconds
randomGuy2 turn left 2 revolutions duration = 2 seconds
randomGuy2 orient to camera duration = 2 seconds
randomGuy2 turn left 0,5 revolutions
randomGuy2.upperBody turn at speed left speed = 2 revolutions per second
randomGuy2.leftLeg.knee turn at speed left speed = 2 revolutions per second
randomGuy2.rightLeg turn at speed right speed = 2 revolutions per second
randomGuy2.leftLeg.knee turn right 2 revolutions
camera get a good look at girl duration = 2 seconds style = abruptly
girl move amount = 1 meter toward target = camera duration = 2 seconds
girl orient to camera duration = 2 seconds style = abruptly
Do in order
light orient to girl duration = 2 seconds
girl orient to camera duration = 2 seconds
Loop 5 times times
girl.leftLeg turn right 2 revolutions duration = 1 second
girl.rightLeg turn right 2 revolutions duration = 1 second
girl.body turn right 0,25 revolutions duration = 1 second
girl.body turn right 0,5 revolutions duration = 1 second
girl.body turn left 0,25 revolutions duration = 1 second
girl.body.neck turn left 0,25 revolutions duration = 1 second
girl.body.neck turn right 0,5 revolutions duration = 1 second
girl.leftLeg orient to camera duration = 2 seconds style = abruptly
girl.rightLeg orient to camera duration = 2 seconds style = abruptly
girl.rightLeg.lowerLeg turn right 2 revolutions duration = 1 second
girl.leftLeg.lowerLeg roll left 2 revolutions duration = 1 second
girl.body.leftArm roll left 2 revolutions duration = 1 second

girl.body.neck.head orient to camera duration = 2 seconds style = gently
girl.body.leftArm.forearm roll left 2 revolutions duration = 1 second
girl.rightLeg roll right 2 revolutions duration = 1 second
girl.body.rightArm.forearm roll right 2 revolutions duration = 1 second
girl.body.neck turn left 0,25 revolutions duration = 1 second
girl turn left 2 revolutions duration = 1 second style = abruptly
light orient to girl
girl orient to camera duration = 1 second style = gently
Do together
randomGuy2 stand up duration = 1 second style = gently
camera get a good look at randomGuy2 duration = 1 second
Do together
camera get a good look at randomGuy2 duration = 2 seconds
randomGuy2 point at girl duration = 2 seconds
camera get a good look at girl duration = 2 seconds
light orient to girl duration = 2 seconds
camera get a good look at girl.body duration = 2 seconds
light orient to girl.body duration = 2 seconds style = gently
girl orient to camera duration = 2 seconds style = gently
Do together
camera get a good look at randomGuy2 duration = 2 seconds
Do in order
randomGuy2 say Hey! duration = 2 seconds
randomGuy2 say You are super! duration = 2 seconds

evilStepSister2
Methods

evilStepSister2.dialog2 () No variables
girl say We are dancing and dancing. duration = 2 seconds

girl
Methods

girl.dialog2 () No variables
girl say We are dancing! duration = 2 seconds

Diagram 2. Party's Code

We explain that in the code, MP3 music was arranged to play for 230 seconds. This time may be changed accordingly. "Sounds" are presented in ALICE as "seldom used properties". However, our experience led us to the conclusion that this kind of attracting pupils in making

videos with ALICE work perfectly and gives many positive results.

3. AN EXAMPLE OF PRESENTING OWN RECORDING

Example 2.

Video Plato.mov may be considered for teachers in middle schools as another possibility to attract pupils in combining programming videos using ALICE and adding own sound, for example singing a song. This exercise in fact is quite difficult for beginners, as one should record its own graphical presentation of a poem (or a song) together with the voice suitably implemented. As our group of girls have practiced that to make a good recording via ALICE of a short poem or a song needs sometimes many tries. During these multiple repetitions, girls usually were able to learn to recite the poem which gave them additional advantage. Especially if the poem or a song was in a foreign language. Expressing the ideas of a poem as a video was another positive experience which gave pupils a possibility to express more fully their emotions via colourful visualization. For some of them it was really a great pleasure to express thoughts in an animation powered with a piece of music or own declamation.



Diagram 3. A photo from video Plato.mov.

Plato's Code
Created by Ewa Graczyńska

World
Events

When 1 is typed
Do: world.metoda1

When 2 is typed

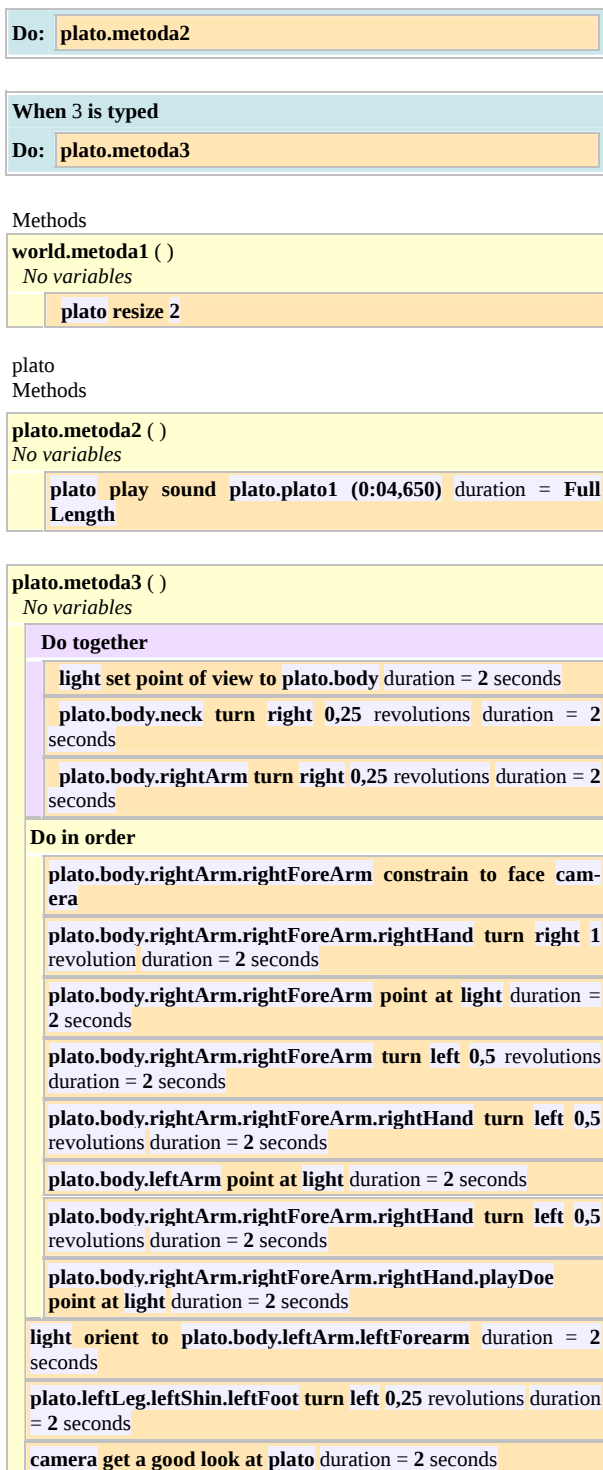


Diagram 4. Plato's Code

4. AN EXAMPLE OF PRESENTING SPECIAL EFFECTS

We studied monograph [1] to find some more possibilities for examples.

Example 3.

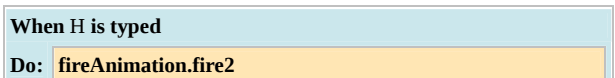
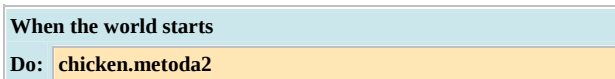
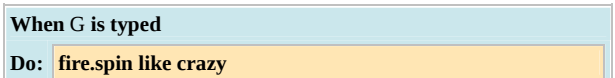
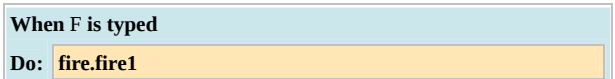
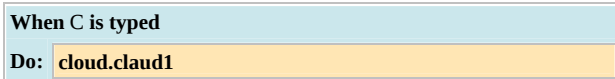
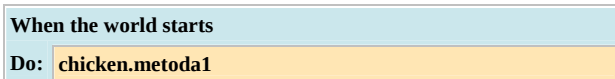
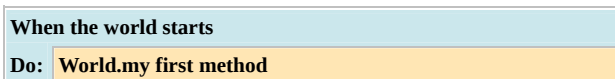
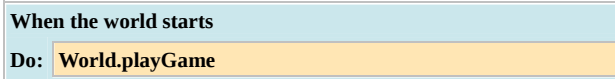
It presents a method of combining implementing sounds with special effects, as fire animations, to attract the presentation, based on Example 1 of [1], p. 307 where a class level variable timeLeft has been created to illustrate how a block of a possible game instructions can be controlled by a timer.

TIMER1's Code

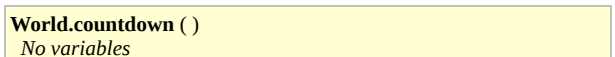
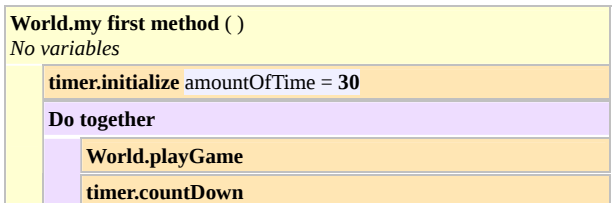
Created by: Ewa Graczyńska

World

Events



Methods



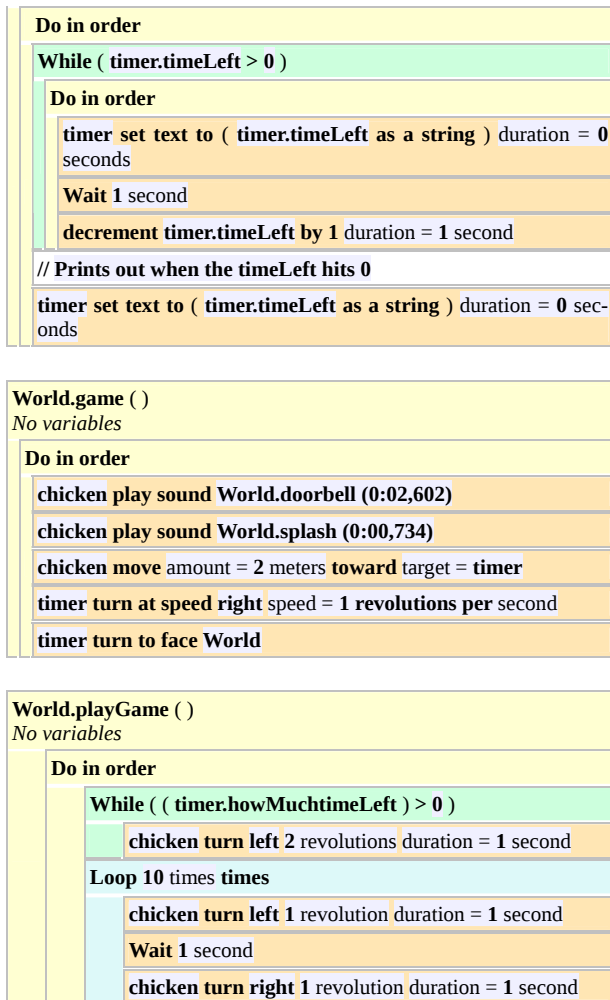


Diagram 5. Timer1'st Code.



Diagram 6. A photo from video Timer1.mov.

We used instructions in Example 1 on p. 307 of [1] to create a timer that counts down in seconds. This exercise was exiting for pupils, as they used some new animations from the CD attached to the monograph [1]. Some pupils were very satisfied with that kind of programming as an early exercise before programming a more complicated game. During our short course of programming in Alice with a use of [1], in the group, the growth of an interest in programming games and also several other subjects was noted. Among them there were foreign languages, especially those in which the MP3 music was listened (English or German) or in which the poems were declaimed. A possibility of a computer visualization of ideas or some natural phenomenon was highly appreciated by the pupils as an additional effect of their experiments.

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