EGE UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

(MASTER OF SCIENCE DEGREE THESIS)

QUALITY CONTROL INVESTIGATION

OF A SKIN MOISTURISER USED IN THE COSMETIC

INDUSTRY, WHICH IS PREPARED

BY THE USE OF OXYGEN-18 HEAVY WATER

INSTEAD OF LIGHT WATER

Çiğdem ÇETİN

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Çiğdem ÇETİN tarafından Yüksek Lisans Tezi olarak sunulan "Quality control investigation of a skin moisturiser used in the cosmetic industry, which is prepared by the use of oxygen-18 heavy water instead of light water" başlıklı bu çalışma, E. Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliği ile E. Ü. Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesi'nin ilgili hükümleri uyarınca, tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve 04 / 07 / 2012 tarihinde yapılan tez savunma sınavında aday oybirliği ile BAŞARILI bulunmuştur.

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ÖZET

OKSİJEN-18 İLE ZENGİNLEŞTİRİLMİŞ SU KULLANILARAK HAZIRLANAN VE KOZMETİK SANAYİNDE KULLANILAN BİR NEMLENDİRİCİNİN NEMLENDİRME KALİTESİNİN İNCELENMESİ

Çiğdem ÇETİN

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Kozmetik sanayinde nemlendiricilerin hazırlanmasında bildiğimiz ^{16}O'lı su ($H_2^{16}O$) kullanılmaktadır. Bunun yanında, nemlendiricilerin içerisindeki suyun ciltte uzun süre kalarak cildi nemli tutabilmesi için, suyun kolay buharlaşmasını engelleyecek ve böylece cilt üzerinde suyun tutulmasını sağlayacak petrokimyasal katkı maddeleri de kozmetik malzemelerin hazırlanmasında kullanılmaktadır. Petrokimyasallar uzun süreli kullanımlar sonrası bazı dokularda birikerek kansere neden olabilmektedir. Deri üzerinde suyun tutulabilmesi için nemlendiricilerde minimum düzeyde petrokimyasal kullanılması durumunda, insan sağlığına yönelik bu risk faktörünün de büyük ölçüde azalacağı kuşkusuzdur. Bu da ancak, suyun doğal yollarla deri üzerinde tutulmasını, diğer bir deyişle, deri üzerinden geç buharlaşmasını sağlamakla mümkün olabilecek bir husustur.

"^{18}O'li Ağır Su" ($H_2^{18}O$), yaygın olarak bulunan Hafif su'ya oranla biraz daha yüksek bir kaynama noktasına, dolayısıyla daha düşük bir buhar basıncına sahiptir ve deri üzerinden buharlaşmasının da Hafif su'ya oranla daha düşük olduğu bilinmektedir. Nemlendiricilerin önemli miktarda $H_2^{18}O$ içermesi durumunda, cilt üzerinde daha uzun süre tutulacağı ve nemlendirme etkisinin artırılması amacıyla kullanılmakta olan bazı petrokimyasal maddelerin kullanılmasına da fazla gereksinim kalmayacağı düşünülmektedir. Bu da, sağlıklı, kaliteli ve cildi nemlendirme etkisi daha yüksek kozmetik ürünlerin hazırlanması anlamını taşımaktadır. İşte, bu çalışmada "^{18}O'li Ağır Su" ile hazırlanan nemlendiricilerin deri üzerindeki nem oranına etkisi incelenmiştir.

Piyasada satılmakta olan bir nemlendiricinin içindeki su buharlaştırma yöntemiyle 41 ^{0}C sıcaklıktaki silikon yağı banyosunda nemlendiriciden uzaklaştırılmıştır. İlk olarak, buharlaştırma ile nemlendiricinin yapısında değişim olup olmadığının incelenmesi amacıyla, suyu alınan kuru nemlendiriciye buharlaştırılan miktarda saf su ilave edilmiştir. Oluşturulan bu yeni nemlendirici ile söz konusu nemlendiricinin el ve kol bölgelerinde verdiği nem ölçüm sonuçları, nemlendiricinin yapısının değişmediğini, nemlendiriciden yalnızca suyun buharlaştırıldığını göstermiştir. Diğer taraftan, oda sıcaklığında buharlaşmaya bırakılan nemlendiriciden buharlaşan su yüzdesinin, silikon yağı banyosunda buharlaştırılan su yüzdesi ile aynı olduğu da tespit edilmiştir.

''^{18}O'li Ağır Su''yun etkisinin incelenmesi amacıyla ise, suyu alınan nemlendiriciye bu defa aynı miktarlarda değişik ^{18}O zenginliğine sahip ''^{18}O'li Ağır Su''lar ilave edilerek bu nemlendiricilerin deri üzerindeki nem oranına etkileri incelenmiştir. ''^{18}O'li Ağır Su'' ile hazırlanan nemlendiricilerin el ve kol bölgelerinde söz konusu nemlendiricinin önceki durumuna göre deriye daha fazla nem verdiği, artan ^{18}O zenginliğinin yükselmesi ile derideki nem oranının da arttığı ve bu etkinin su ile temas olmadığı sürece 24 saat kadar devam ettiği tespit edilmiştir.

Elde edilen bu sonuçlar, ''^{18}O'li Ağır Su'' ile üretilebilecek yeni nemlendiricilerin deriyi oldukça daha uzun süre nemli tutabileceğini ortaya koymuş bulunmaktadır ki bunun da, kozmetik sanayi için oldukça önemli olduğu düşünülmektedir.

Anahtar sözcükler: İzotop, Kararlı izotop, Oksijen-18, ^{18}O'li ağır su, Nemlendirici, Kozmetik sanayi, Petrokimyasal ürünler.

ABSTRACT

QUALITY CONTROL INVESTIGATION OF A SKIN MOISTURISER USED IN THE COSMETIC INDUSTRY, WHICH IS PREPARED BY THE USE OF OXYGEN-18 HEAVY WATER INSTEAD OF LIGHT WATER

Çiğdem ÇETİN

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For the preparation of moisturisers in the cosmetics industry, light water ($H_2{}^{16}O$) is being used. In order to imprison the water molecules inside the moisturiser, petrochemical additives that make water evaporate harder are used on the skin. These petrochemicals might accumulate after long-term use and cause cancer. If the use of petrochemicals to keep water molecules on the skin was kept to a minimum, the risks to human health would be minimised. However, this is only possible if natural ways to keep water on the skin longer and evaporate later are provided.

In comparison to base water, "Heavy ^{18}O Water" has a higher boiling point, and therefore, less vapour pressure, so it evaporates less and later from the skin. In the moisturisers that contain "Heavy ^{18}O Water", more water will remain on the skin for longer so that there will be no need for petrochemicals to increase the moisturising effect. Thus, this means a higher quality and higher moisturising ratio for cosmetics. The aim of this study, is to investigate the effects of the moisturisers prepared with ''Heavy ^{18}O Water'' on the moisture ratio of the skin.

The water inside a moisturiser from the market was evaporated at 41°C in a silicone oil bath. First, pure water was added in the same amount to investigate whether the character of the moisturiser had changed. Moisture measurements on the arms and hands to compare the new moisturiser prepared with pure water and the moisturiser from the market showed that the characteristics of the moisturiser

remained undisturbed. Other moisturisers left at room temperature to evaporate and those in the oil bath lost the same amount of water.

''Heavy ^{18}O Water'' was then added in the same amount, but with different ratios of ^{18}O, to the evaporated moisturiser and the effects on the moisture ratio of the skin were investigated. The moisturisers prepared with ''Heavy ^{18}O Water'' showed more moisture compared to the ones from the market, and the ratio of moisture was consistent with the amount of ^{18}O in the water. Additionally, if not contact was made with water, the effect lasted for about 24 hours.

In the light of the obtained results, cosmetic moisturisers that are produced with "Heavy ^{18}O Water" can keep the skin moisturised for longer, which carries a significant importance for the cosmetics industry.

ACKNOWLEDGEMENT

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1. INTRODUCTION

1.1 General Information about Stable Isotopes and the ^{18}O Isotope

1.1.1 Atomic nucleus and isotopes

In this section, information about an atomic nucleus and its structure, the stability of an atomic nucleus, stable and radioactive isotopes, and natural isotopic abundances of elements are given.

1.1.1.1 <u>Atomic nucleus and structure</u>

An atomic nucleus which is confined within electron clouds is at the centre of an atom. The nucleus contains protons and neutrons in addition to many other elementary particles. Thus, the mass of nucleus primarily comes from the mass of the protons and neutrons. Protons and neutrons are called nucleons. The protons carry a positive electric charge while the neutrons have no electric charge. Each nucleon is formed with three different quarks. The electrons have a negative charge and their mass is much smaller than that of both the protons and neutrons (Ünak, 2006).

The nucleus is the heaviest part of an atom, constituting 99.9 % of the total atomic mass and occupying a volume whose radius is $1/100,000^{th}$ of the size of the atom.

Gravity, the electromagnetic force, the weak force and the strong force are four different ways in which matter affects other matter. The strong and weak forces are short range forces that are effective only within the nuclei of atoms. The strong force binds together atomic nuclei, whilst the weak force is involved in radioactive decay and other interactions of certain kinds of nuclear particles (Backman and Seeds, 2012).

1.1.1.2 <u>The stability of an atomic nucleus</u>

The stability of an atomic nucleus means that the atomic nucleus does not spontaneously emit any radiation. The dominant factor of nuclear stability is the neutron to proton (N / P) ratio. This ratio is close to 1 for atoms of elements with low atomic numbers and increases as the atomic number increases (Ünak, 2006).

A plot of the numbers of protons (Z) and neutrons (N) in stable and unstable nuclides is shown in Figure 1.1.1.2 (Tutor Vista, 2012).

Nuclides containing even numbers of both protons and neutrons are more stable than nuclides containing odd numbers of protons and neutrons. Nuclides containing an odd number of protons and an even number of neutrons are less stable than nuclides containing even numbers of protons and odd numbers of neutrons. The Odd-Even factor in nuclear stability is shown in Table 1.1.1.2.
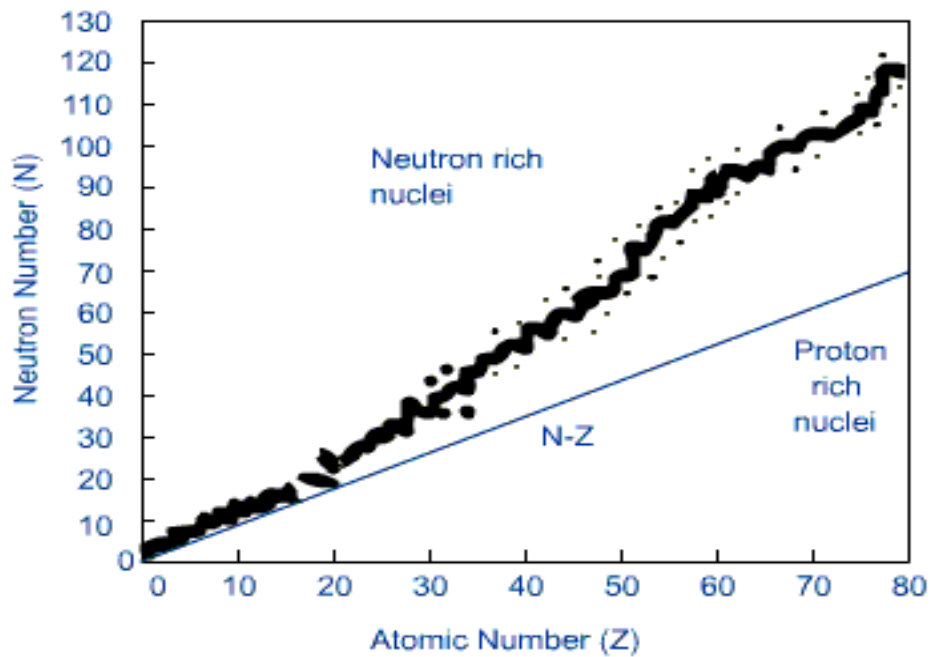
Figure 1.1.1.2 Plot between numbers of protons (Z) and neutrons (N) of stable and unstable nuclides.

Table 1.1.1.2 Odd-Even factor in nuclear stability.

Protons	Neutrons	Number of Stable	Nuclides Stability
Odd	Odd	4	least stable
Odd	Even	50	↓
Even	Odd	57	↓
Even	Even	168	most stable

1.1.1.3 <u>Stable and radioactive isotopes</u>

Atoms of the same element with the same atomic number but a different number of neutrons are called isotopes.

Elements can be found in stable or non-stable (radioactive) forms in nature and usually have two or more stable isotopes. However, one isotope is generally found much more frequently than others (Rundel et al, 1989).

The atomic nuclei of stable isotopes are stable, whilst radioactive isotopes cause radioactive decay. The nuclei of radioactive atoms are heavy and can break apart easily.

Today, new and useful radioisotopes are produced by bombarding a target element with high speeds in a cyclotron (Jespersen, 2010).

Radioisotopes are important for medical imaging and radiation therapy, as well as reaction path tracers in medical, biological, agricultural and, chemical research sciences (Davisson, 2002).

1.1.1.4 <u>Natural isotopic abundances of elements</u>

The isotopic abundance is the relative number of atoms of a special isotope in a mixture of the isotopes of a chemical element, which is denoted as a fraction (%) of all of the atoms of the element.

The isotopic abundances of the elements vary depending on the source of the materials. The natural isotopic abundance is the isotopic abundance in the element as found on earth. However, because of natural radioactivity, this amount may not be well defined for some isotopes (Vértes et al., 2003).

An element generally occurs in an isotope mixture, with one isotope in the mixture being more predominant than the others. For example, ^{16}O, ^{17}O, and ^{18}O are stable isotopes of oxygen.

Hydrogen has two stable isotopes: ^{1}H and ^{2}H (deuterium, D) with natural abundances of 99.985 % and 0.015 %, respectively. ^{18}O and ^{2}H are often useful in hydrological studies, as they allow the direct tracing of water pathways.

In soil water studies, the isotopes of interest are the isotopes used in studies of water itself (isotopes of oxygen and hydrogen) or those of associated elements that are dissolved in water (Gliński et al., 2011).

1.1.2 Oxygen-18 (^{18}O)

In this part, knowledge of oxygen and its stable and radioactive isotopes, the natural abundances of oxygen isotopes, the oxygen-18 (^{18}O) isotope and its natural distribution, natural enrichment of the ^{18}O isotope, ^{18}O variations in certain natural samples, literature on ^{18}O measurements, the use of ^{18}O in science and technology, and enrichment procedures of ^{18}O are given.

1.1.2.1 <u>Oxygen and its stable and radioactive isotopes</u>

Three stable isotopes occur in oxygen, which are ^{16}O, ^{17}O, and ^{18}O. Giaque and Johnston discovered the oxygen isotopes ^{17}O and ^{18}O in 1929. The dispersion of the stable isotopes are: ^{16}O = 99.759 %, ^{17}O = 0.037 %, and ^{18}O = 0.204 %.

All three Oxygen isotopes are used in medical applications. ^{16}O is used in the production of radioactive ^{13}N, which is used for positron emission tomography (PET) imaging and myocardial perfusion. ^{17}O can be used as a tracer in the study of cerebral oxygen utilisation. ^{18}O is used for the production of ^{18}F, which is used as a tracer in PET. FDG-PET medical procedures are performed to investigate diseases in various human organs (WebElements, 2012).

On the other hand, oxygen has twelve other radioisotopes recently identified: ^{12}O, ^{13}O, ^{14}O, ^{15}O, ^{19}O, ^{20}O, ^{21}O, ^{22}O, ^{23}O, ^{24}O, ^{25}O, and ^{26}O, which are shown in Table 1.1.2.1.

Table 1.1.1.5 Radioisotopes of oxygen.

clide symbol	Z(p)	N(n)	isotopic mass (u)	half-life	decay mode(s)[14]	daughter isotope(s)[n 1]
^{12}O	8	4	12.034405(20)	$580(30)\times10^{-24}$ s [0.40(25) MeV]	2p (60.0%)	^{10}C
					β^+ (40.0%)	^{12}N
^{13}O	8	5	13.024812(10)	8.58(5) ms	β^+ (89.1%)	^{13}N

					β^+, p (10.9%)	[12]C
[14]O	8	6	14.00859625(12)	70.598(18) s	β^+	[14]N
[15]O	8	7	15.0030656(5)	122.24(16) s	β^+	[15]N
[19]O	8	11	19.003580(3)	26.464(9) s	β^-	[19]F
[20]O	8	12	20.0040767(12)	13.51(5) s	β^-	[20]F
[21]O	8	13	21.008656(13)	3.42(10) s	β^-	[21]F
[22]O	8	14	22.00997(6)	2.25(15) s	β^- (78.0%)	[22]F
					β^-, n (22.0%)	[21]F
[23]O	8	15	23.01569(13)	82(37) ms	β^-, n (57.99%)	[22]F
					β^- (42.0%)	[21]F
[24]O	8	16	24.02047(25)	65(5) ms	β^-, n (57.99%)	[23]F
					β^- (42.01%)	[24]F
[25]O	8	17	25.02946	5.2×10^{-8} s	N	[24]O
[26]O	8	18	26.03834	4.0×10^{-8} s	β^-	[26]F
					N	[25]O

1.1.2.2 <u>Natural abundances of Oxygen isotopes</u>

As mentioned before, oxygen has three stables isotopes: ^{16}O (99.76 %), ^{17}O (0.038 %) and ^{18}O (0.204 %). Oxygen isotope ratios are generally measured as CO_2 or CO using a gas source for Isotope Ratio - Mass Spectrometry (IR-MS). Variations in oxygen isotope abundance are compared to the Vienna Standard Mean Ocean Water (VSMOW) international standard (Coplen et al., 1983).

The abundance ratios of oxygen stable isotopes are as follows: ^{17}O / ^{16}O = 1 / 2700, ^{18}O / ^{16}O = 1 / 490. These ratios are variable in natural materials due to a variety of physical and chemical processes, ranging from stellar nucleosynthesis to ordinary mass dependent chemical fractionation.

Chemical and physical processes within an isolated planetary body, such as the earth, moon and Mars almost always obey a simple mass dependent relationship between variations in ^{17}O / ^{16}O and ^{18}O / ^{16}O ratios:

$$d\delta\ ^{17}O\ /\ d\delta^{18}O \simeq 0.52$$

Meteorite studies have shown that oxygen isotope abundance variations between solar system members are governed principally by a different systematic relationship:

$$d\delta\ ^{17}O\ /\ ^{18}O \simeq 1.0$$

In this delta notation, isotope ratios are given relative to a standard reservoir. For solar system studies, the ideal standard would be the sun itself, representing the reservoir from which planetary bodies were formed. But the solar isotopic composition is not yet known with sufficient accuracy for that purpose (Kallenbach and Geiss, 2003).

^{18}O is generally preferred to ^{17}O in environmental studies, because it is easier to measure as it is more abundant.

Molecular oxygen in the atmosphere has an isotope composition of +24 ‰ with respect to SMOW. Water at a mid-latitude location may have an oxygen isotope composition of -10 ‰ with respect to the same standard.

Oxygen isotopes in precipitation vary with latitude, altitude, and with distance inland from a coastal site and are a function of the isotope compositions of the source water, the relative humidity and the temperature at which evaporation took place (Dansgaard, 1964).

1.1.2.3 Oxygen-18 isotope and its natural distribution

The oxygen isotope composition of phytolith silica is depended on soil water $\delta^{18}O$ values, relative to evapotranspiration, humidity, and temprature during plant growth (Webb et al., 1999).

The $\delta^{18}O$ of precipitation is also influenced by temperature, altitude, distance inland along different stormtracks, environmental conditions at the source of the vapour, latitude, and humidity. The delta values are usually more positive in the summer and more negative in the winter, and decrease with increasing altitudes because of seasonal temperature differences (Kendall et al., 2001).

There are usually more of these heavy isotopes in leaf water than in the soil / xylem water, because the vapour pressure of heavy water is less than that of the most common isotopologue, $H_2^{16}O$, and the binary diffusivity with air of heavy water vapour is less than that of light water. Therefore, when water evaporates from the leaf, heavier molecules are left behind and this process continues until the leaf water becomes sufficiently enriched such that the exit of heavy and light molecules through the stomata matches that of the supply of water from the xylem (Farquhar et al., 2007).

1.1.2.4 Natural enrichment of Oxygen-18 isotope

Depending on the temperature of evaporation, the weight ratio ^{16}O / ^{18}O ($\delta^{18}O$ value), in water vapour is a few percent higher than in sea water. The isotopic composition of water vapour in the atmosphere is, therefore, not the same as that of sea water; atmospheric water vapour includes more of the ^{16}O isotope, whereas sea water is enriched with the ^{18}O isotope.

Water vapour enriched with ^{16}O is transported by wind in the atmosphere from the sea to land. When the water vapour condenses and precipitates as rain, snow or hail, the water becomes rich in ^{16}O. Eventually, the ^{16}O rich water is incorporated into rivers, lakes and glaciers, but this is not the same as in seas and oceans.

Leaf water $\delta^{18}O$ values are typically enriched in ^{18}O compared with the plant's source water. That is because the evaporative losses of water from the leaves are greater for $H_2^{16}O$ than for $H_2^{18}O$ (Kahmen et al., 2008).

1.1.2.5 Oxygen-18 variations in some natural samples

Stable isotopes of an element are denoted as a ratio of the scarce isotope to the abundant isotope, as it is easier to measure isotopic ratios, rather than absolute values. Isotope ratio analysis is performed using isotope ratio mass spectrometry. Recently, other successful spectroscopic techniques have been developed.

In natural abundance studies, isotope ratios are reported, referenced against international standards, in delta (δ) units, parts per thousand (‰). This is also called the isotopic signature. As mentioned before, variations in oxygen

isotope abundance are compared to the VSMOW international standard (Gliński et al., 2011).

$$\delta^{18}O_{sample} = \left(\frac{\left(\frac{^{18}O}{^{16}O}\right)_{sample}}{\left(\frac{^{18}O}{^{16}O}\right)_{VSMOW}} - 1 \right) * 1000$$

The method for measuring oxygen - isotopic composition involves dissolved sulphate ions and barium sulfate. Sulphate ions in oceans are enriched in ^{18}O by 9.5 per mille relative to mean ocean water and show only minor variations, probably because the exchange rate is slow enough to prevent local equilibrium with surface waters.

Sulphate ions in saline lakes and brines have an ^{18}O enrichment of 7 to 23 ‰ relative to mean ocean water. The value of the highest enrichment observed is about the same as that of atmospheric oxygen (Longinelli and Craig, 1967).

The results of deuterium and ^{18}O measurements on microlitre samples of urine, plasma, saliva, and human milk were described that enriched levels of ^{2}H (580 ‰) and ^{18}O (256 ‰) can be measured with a precision of 3.2 ‰ and 0.97 ‰ and an accuracy of -4.6 ± 4.4 ‰ and -0.32 ± 0.87 ‰, respectively (Wong et al., 1987).

The isotopic composition of body water in mammals has been shown to track the isotopic composition of their environmental water, although the relationship varies from one mammal species to another. The $\delta^{18}O$ of local precipitation varies seasonally and the isotopic signature may be modified by other mechanisms, primarily evaporation, as a function of geographic location (Yurtsever et al., 1981; Ayliffe et al., 1990).

Marine carbonates are usually expressed relative to the VPDB scale, which differs by about 30 ‰ from the VSMOW scale (Coplen, 1994).

The oxygen isotope composition of phytolith silica is dependent on soil water $\delta^{18}O$ values, relative humidity and evapotranspiration, as well as temperature during plant growth. The $\delta^{18}O_{silica-plant\ water}$ remained constant at

~ 34 ‰ and the $\delta^{18}O$ values of plant water collected from pre-dawn and midday samplings showed little variation. Phytoliths at all three sites have a similar pattern of $\delta^{18}O$ values within the plants, but the isotopic separation between leaf and stem silica increases from 4 to 8 ‰ as average relative humidity decreases (Webb and Longstaffe, 1999).

Mass independent $\delta^{18}O$ isotopic variation has been observed for O_3, O_2, N_2O, H_2O_2, CO, and CO_2 and is attributed to the discrimination between symmetric and asymmetric isotopic species during photochemical reactions (Thiemens et al., 1995; Thiemens, 2001; Gao et al., 2001).

1.1.2.6 Literature on Oxygen-18 measurements

Carbonate and phosphate $\delta^{18}O$ values have been used extensively in oxygen isotope paleotemperature studies (Epstein, 1953; Longinelli et al., 1968).

In carbonates, the determination of ^{18}O is carried out by treating the accepted fractionation factors of the oxygen content of water (McCrea et al., 1950; Fontes et al., 1965).

The lowest $\delta(^{18}O)$ value found in the literature for a naturally accouring material is 62.8 ‰ for Antarctic precipitation (Aldaz et al., 1967).

The ^{18}O / ^{16}O ratio in cellulose allows the reconstruction of the climatic conditions present during biosynthesis in plants (Epstein et al., 1977).

The $\delta(^{18}O)$ of bulk Earth is well represented by mid-ocean ridge basalts ($+5.7 \pm 0.5$ ‰) which cover much of the ocean flor (Kyser et al., 1986).

The determination of oxygen isotope ratios of inorganic anions is an important means for the elucidation of geo- and biochemical, agricultural, and environmental questions such as mechanisms of biological reactions (Cohn et al., 1995; Schmidt et al., 1991), the nature and extent of biological transformations (Böttcher et al.1990), and the origin assignment of the compounds (Amberger et al., 1987; Aravena et al., 1992).

A method for the automated sample conversion and on-line oxygen isotope ratio $\delta^{18}O$ determination for organic and inorganic substances is presented (Kornexl et al., 1999).

1.1.2.7 <u>Use of Oxygen-18 in science and technology</u>

The ratio of ^{18}O / ^{16}O isotopes in a compound can help to identify its oxygen source and possibly also the processes in which it was involved. Therefore, methods for the determination of $\delta^{18}O$ values of any substance are important tools in anthropology, biochemistry, geology, ecology, food chemistry, medicine, and many other fields.

^{18}O is used to as a starting material for the medical radioisotope ^{18}F for PET, as a tracer during medical and biological research, creating therapeutic cancer drugs, and some environmental studies (Center of Molecular Research, 2012).

2-Fluoro-2-deoxy-D-glucose with ^{18}F radiolabeled (^{18}FDG) is an analogue of glucose which are used to explore the possibility of cancer in nuclear medicine imaging. Water which has been enriched with oxygen-18 isotopes is the target material of ^{18}FDG.

A proton which has high energy interacts with the nucleus of ^{18}O to produce ^{18}F. A high energy neutron is emitted and the ^{18}F isotope formed (Jadvar and Parker, 2005).

$$^{18}O \ (p, \ n) \ ^{18}F$$
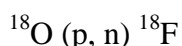
^{18}O is a useful tracer of the origin and timing of groundwater recharge (Winograd et al., 1988; Lee et al., 1999; Barth, 2000), which is the other application detecting of food adulteration (Kelly et al., 2005).

The ratio ^{18}O / ^{16}O ($\delta^{18}O$) can be used to determine paleothermometry in certain types of fossils.

Some studies are present which have been made online measurements of oxygen-18 abundances by coupling an elemental analyser or a gas chromatograph to an Isotope Ratio - Mass Spectrometer (Kornexl et al., 1999).

1.1.2.8 <u>Enrichment procedures of Oxygen-18</u>

Widely used enrichment technique for oxygen-18 is column distillation technique. The separation of $H_2^{16}O$ and $H_2^{18}O$ occurs in distillation columns. A cube-evaporator, the actual columns, a filled nozzle, a condenser, and the upper reservoir are located in each column. In the cube, there is a continuous evaporation of water from the column. Steam rising up the column interacts with the water flowing as a film on the nozzle, mass transfer occurs, the steam is depleted, and the flowing water is enriched with a high boiling component $H_2^{18}O$. At the top of the column, steam entered into the condenser, cooled by water from a water recycling system. In the condenser is a continuous condensation of depleted oxygen-18 water vapour. Gravity condensate enters the upper reservoir and mixes with water located there. After this, the water is fed into the power of the column. The consumption of water corresponds to the mass flow of steam. Thus, during operation, the process of the column is to transfer oxygen-18 from the upper reservoir to the cube; water in the cube is enriched in oxygen-18 and the water in the upper reservoir is depleted of this isotope (Centre of Molecular Research, 2012).

The rectification technique is prevalently used for the separation of substances with different boiling points. Natural water is acceptable as a mixture of the low-boiling component $H_2^{16}O$, with a boiling point of 100 °C at normal pressure, and the high-boiling component $H_2^{16}O$, with a boiling point of 100.15 °C.

Another technique for the enrichment of ^{18}O is laser light. In this method, a photochemical reaction induced by laser light is employed to separate oxygen isotopes. A solution containing UO_2F_2, HF, H_2O and a large excess of CH_3OH is irradiated with laser light of an appropriate wavelength to differentially excite the UO^{22+} ions containing ^{16}O atoms and cause a reaction to proceed in accordance with the following reaction:

$$2\ UO_2\ F_2 + CH_3OH + 4\ HF \rightarrow 2\ UF_4 \downarrow + HCOOH + 3\ H_2O$$
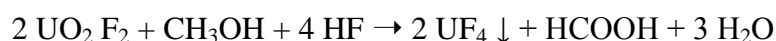
Irradiation is discontinued when about 10 % of the UO_2F_2 has reacted. The UF_4 is filtered from the reaction mixture and the residual CH_3OH and HF, plus the products HCOOH and H_2O, are distilled away from the UO_2F_2, which is thereby enriched in the O^{18} isotope. Also, the solution containing the UO_2F_2 may be

photochemically processed again to provide further enrichment of the ^{18}O isotope (De Poorter and Rofer-De Poorter, 1975).

1.1.3 Oxygen-18 water

In this part, knowledge of oxygen-18 enriched water and its economical potential are given.

1.1.3.1 <u>Oxygen-18 enriched water</u>

The existence of the three isotopes gives rise to three slightly different molecules of water, with each molecule containing a different isotope of oxygen: $H_2^{16}O$, $H_2^{17}O$, $H_2^{18}O$. Since the oxygen isotopes are of different weights, there are differences in the weight of the molecules of water that they form.

Evaporation of the lighter isotope requires less energy, thus, water has a lower oxygen-18 to oxygen-16 ratio in the atmosphere. Whenever water evaporates, there is preferential evaporation of the lightest water molecules, including oxygen-16 and an increase in the concentration of water molecules containing the heavier isotope, oxygen-18.

In the mid-1990s the supply of oxygen-18 enriched water was tenuous, whilst, recently, there has been a more abundant supply of oxygen-18 enriched water (Jadvar and Parker, 2005).

1.1.3.2 <u>Economical potential of Oxygen-18 enriched water</u>

Oxygen-18 as a stable isotope of oxygen is obtainable in the form of ^{18}O water ($H_2^{18}O$). ^{18}O is important for the production of 2-fluoro-2-deoxy-D-glucose radiolabeled with ^{18}F (^{18}FDG) used in Positron Emission Tomography (PET). which is an imaging technique in nuclear medical applications. In the radiopharmaceutical industry, $H_2^{18}O$ is bombarded with hydrogen ions in either a cyclotron or a linear accelerator, generating ^{18}F. This is then synthesised into ^{18}FDG and injected into a patient (Isoflex, 2012).

$H_2^{18}O$ has very important application potential in the cosmetic sector, which aims to produce healthier cosmetic materials because ^{18}O is evaporated

more slowly with respect to regular water. This results in keeping the skin more hydrated, which means clearer, healthier, and more brilliant skin.

Because of these kinds of applications, ^{18}O has an important economic potential.

1.2 General Information about Cosmetic Products

1.2.1 The historical development of using of cosmetic products

The history of cosmetics and personal care products started in ancient times. Ancient Egyptians used perfumes and oil for the body as early as 4000 BC. Egyptians, Greeks, and Romans made cosmetics from minerals and plants for their face, hair design, and eye make up.

Africans painted their bodies for war and magical ceremonies. In North America, animal fats were applied as a base for body paint and for protection against insects and the cold (Singh, 2008).

By the early 1900s, most people only used basic cosmetics, such as face powder, rouge, and shampoo. The 19th and 20th centuries have developed other products and techniques of manufacture, packaging and advertising, which have revolutionised the industry and made products available on an unprecedented scale (Singh, 2008).

By the mid-1920s cosmetic firms ranked second behind food companies in the amount of money spent on advertising and they expanded the percentage of their budgets spent on advertising over the course of the twentieth century (Smith, 2008).

1.2.2 Current cosmetic product categories

The cosmetic product categories are listed by Federal food, Drug, and Cosmetic Act, USA (FDA) as given in the Table 1.2.2.

Table 1.2.2 The cosmetic product categories.

Classification	Products
Baby products	Baby Shampoos, lotions, oils, powders, and creams, other baby products.
Bath preparations	Bath oils, tablets, salts, bubble bath, bath capsules, other bath preparations.
Eye makeup preparations	Eyebrow pencil, eyeliner, eye shadow, eye lotion, eye makeup remover, mascara, other eye makeup preparations.
Fragrance preparations	Cologne and toilet water, perfumes, powders (dusting and talcum) sachets, other fragrance preparations.
Hair preparations (non-colouring)	Hair spray (aerosol fixatives), hair straightners, permanent waves, rinses (non-colouring), shampoos (non-colouring), tonics, dressings, and other hair grooming aids, wave sets, other hair preparations
Hair colouring preparations	Hair dyes and colours (all types requiring caution statements and patch tests), hair tints, hair rinses (colouring), hair shampoos (colouring), hair colour sprays (aerosols), hair lighteners with colour, hair bleaches, other hair colouring preparations.
Makeup preparations (not eye)	Blushers (all types), face powders, foundations, leg and body paints, lipstick, makeup bases, rouges, makeup fixatives, other makeup preparations.
Manicure preparations	Basecoats and undercoats, cuticle softeners, nail creams and lotions, nail extenders, nail polish and enamel nail polish, enamel removers, other manicure preparations.
Oral hygiene products	Dentifrices (aerosol, liquid, pastes, powders), mouthwashes and breath fresheners (liquids and sprays), other oral hygiene products
Personal cleanliness	Bath soaps and detergents, deodorants (underarm), douches, feminine deodorants, other personal cleanliness products
Shaving preparations	Aftershave lotion, beard softeners, men's talcum powder, pre-shave lotions (all types), shaving cream (aerosol, brushless, and lather), shaving soap (cakes, sticks, etc.), other shaving preparations.
Skin care preparations (Creams, Lotions, Powders, and Sprays)	Cleansing (cold creams, cleansing lotions, liquids, and pads), depilatories, face and neck (excluding shaving preparations), body and hand (excluding shaving preparations), foot powders and sprays, moisturising, night, paste masks (mud packs), skin fresheners, other skin care preparations
Suntan preparations	Suntan gels, creams, liquids, indoor tanning preparations, other suntan preparations.

1.2.3 Ingredients of cosmetic products

As a result of the increase in the use of cosmetic products, an increasing number of new cosmetic products have appeared on the market with new or improved properties. This means that a great many substances are employed in cosmetic products worldwide.

''There are a large number of possible cosmetic products formulated from a great number of raw materials (more than 4000 ingredients are listed in the Cosmetic, Toiletry and Fragrance Association Dictionary and in the Inventory of the European Union), so a complete cosmetic formulation can contain up to 40 different inactive chemicals and one or more pharmacologically-active components (Baran, 2005).''

The category of cosmetic ingredients is listed below (The Cosmetics Cop).

1.2.3.1 Absorbent

Absorbents are substances which are added to cosmetic products to take up water and soluble oil in dissolved or finely dispersed substances.

1.2.3.2 Antibacterial / anti-acne

This group reduces the chances of inflammation and infection of the pores by killing the bacteria.

1.2.3.3 Anti-irritants

Anti-irritants are widely used in cosmetic formulations, with the aim of reducing irritation from substances in the formulation.

1.2.3.4 Antioxidants

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals.

1.2.3.5 Cell-communicating ingredients

This group reduces cellular damage that leads to destruction of the skin's supportive elements, thus preventing significant wrinkles.

1.2.3.6 Colouring agents / pigments

The colour of a tangible object is the result of pigments or molecular colouring agents.

1.2.3.7 Emollients

Emollients, also commonly referred to as moisturisers, are products that help to soften the skin or to treat skin that has become dry.

1.2.3.8 Emulsifiers

An emulsifier is the ingredient which binds the water and oil in a cream or lotion together permanently.

1.2.3.9 Film-forming / holding agents

Film-forming agents can be weak skin sensitizers.

1.2.3.10 Fragrance: Synthetic and fragrant plant extracts

Plant extracts and essential oils are natural additives to make perfume smell.

1.2.3.11 Irritants

Some ingredients in cosmetic products can cause allergic reactions or irritation in some users.

1.2.3.12 Leave - on exfoliant

This group is used to remove dead skin cells with a regular exfoliating scrub.

1.2.3.13 Miscellaneous

Miscellaneous ingredients are used to enhance the appearance of dry or damaged skin by reducing flaking and restoring suppleness.

1.2.3.14 Plant extracts

Plant extracts and essential oils are natural additives to make perfume smell.

1.2.3.15 Prescription drugs

Over-The-Counter drugs are usually regulated by active pharmaceutical ingredients (APIs) and cannot be purchased without a doctor's prescription.

1.2.3.16 Preservatives

Preservatives extend a cosmetic product's life and help to prevent the growth of bacteria; but, they can also cause allergic reactions.

1.2.3.17 Scrub agents

Scrub agents help to remove dead skin cells.

1.2.3.18 Silicones

These ingredients are used to remove undesirable changes, such as wrinkles and uneven pigmentation.

1.2.3.19 Skin-identical ingredients

Skin-identical ingredients are produced entirely of body and skin and essential vitamins. These ingredients provide maximum protection and a healthy, youthful looking skin and also remove existing toxins.

1.2.3.20 Slip agents

Slip agents help other substances to flow more easily and more smoothly without reacting chemically.

1.2.3.21 Sunscreen actives

This group of ingredients is applied to the skin to absorb, reflect, or scatter UV rays.

1.2.3.22 <u>Surfactants / detergent cleansing agents</u>

Cleaning products may also contain enzymes that degrade protein-based stains, bleaches to de-colour stains and add power to cleaning agents, and blue dyes to counter yellowing.

1.2.3.23 <u>Thickeners</u>

Thickeners supply volume and viscosity of cosmetic products, thereby providing more stability and a better performance for product

1.2.3.24 <u>Vitamins</u>

Vitamins supply the skin with the suppression of pigmentation, bruising, and stimulation of collagen synthesis, as well as the refinement of the skin surface and antioxidant and anti-inflammatory effects.

1.2.4 Cosmetic chemistry and its applications

Cosmetic chemistry for the skin treatment professional explores the ingredients most usually found in modern cosmetic products, explains their actions and roles in the formulations and relates them to the effect they may have on specific conditions of the skin. Cosmetic chemistry deals with materials and mixtures for washing, personal hygiene and beauty care, body decoration, and dental care.

The use of cosmetic products is on the increase around the world. On the other hand, all topical products are not cosmetics. For example, dermatological products are topical products generally regulated as drug products based on the therapeutic or medicinal purpose for which the product is marketed, as well as its formulation, which includes one or more pharmacologically-active ingredients (Singh, 2008).

A drug is able to prevent or treat a disease whilst cosmetics are used to clean, protect, and change the appearance of the human body, keeping them in good condition. A cosmetic must not affect the structure and function of skin. Thus, a drug is a medicine for disease and a cosmetic is used for pleasure (Kligman, 1993).

Some products are considered as cosmetics which are Over-The-Counter (OTC) drugs. OTC drugs are usually regulated by active pharmaceutical ingredients (APIs) and cannot be purchased without a doctor's prescription.

Some products can qualify as both cosmetics and OTC drugs when they have two intended uses. For example, an anti-dandruff shampoo is both, because a shampoo is a cosmetic to clean the hair, whilst an anti-dandurff shampoo is a drug for dandruff (Hanson et al., 2011).

The final group in cosmetics includes "natural and organic products". Today, natural cosmetics are the strongest growing market for skin and beauty care around the world. Natural cosmetics serve to beautify and care for the human body with ingredients from nature, meaning that they are friendly to both the skin and the environment. Today, over 50 natural cosmetic brands are known with their ingredients composition. The brands were rated as certified organic, pure natural cosmetic, semi-natural, naturally revealing or traditional cosmetic (Organic Monitor, 2012).

1.2.5 Today's global cosmetic industry

The cosmetic and personal care industry is one of the fastest developing industries today. The skin and hair applications that are prevalently referred to as cosmetics, which include everything from shampoos and soaps to moisturisers and makeup, are products of chemistry. Selecting from more than 5,000 different ingredients, each with its own essential function, cosmetic chemists combine water, colour, oils, emulsifiers, thickeners, preservatives, and fragrances in different ways to produce formulations designed for different purposes.

"According to the data provided by Industrial Technology Research Institute of Taiwan, the market of cosmetics is about 56,500,000,000 NT dollars in 2002. The cosmetics industry is also included in the main properties implemented by the government" (Chen et al, 2011).

"The total EU27 cosmetics market was valued at € 63.5 billion in 2006, compared to € 38.2 billion in the U.S., € 23.7 billion in Japan, and € 8.2 billion in China. Among the EU countries, Germany has the largest cosmetics market, valued at € 11.7 billion, followed by France (€ 10.4 billion), the U.K. (€ 10 billion), Italy (€ 8.8 billion), and Spain (€ 7.4 billion)" (Global Insight, 2007).

"The data on per capita cosmetics spending by country reveal that the average among the EU 27 countries is € 128 per year. Denmark and Sweden have the highest consumption of cosmetic products at € 171, followed by Spain at € 169 and France at € 166. Spending per capita is low in Greece and Portugal at just € 121 and € 104, respectively. Over the period 2000-06, there was sluggish per capita consumption growth

in two of the large European markets, France and Germany, and somewhat better performance in Italy and the United Kingdom. In contrast, there was strong growth in the high per capita consumption countries like Denmark, Sweden, Spain, and Norway. Using a correction for purchasing power parity, per capita cosmetics spending was € 174 in Japan, € 127 in the U.S. and € 24 in China'' (Global Insight, 2007).

The cosmetic industry has grown in the United States, as well as in France, Germany, Italy and Japan. In Germany, the cosmetic industry generated sales of about EUR 12.6 billion in retail sales in 2008, which made the German cosmetic industry the 3rd largest in the world, after Japan and the United States. The exports of Germany in this industry reached EUR 5.8 billion in 2008, whereas the imports of cosmetics totalled EUR 3 billion. After the United States, Japan is the second largest market for cosmetics in the world, a market that is worth about JPY 1.4 trillion per year. The worldwide cosmetic and perfume industry currently generates an estimated annual turnover of US$ 170 billion (according to Eurostaf - May 2007). Europe is the leading market, representing approximately € 63 billion, while sales in France reached € 6.5 billion in 2006. The Italian cosmetic industry plays an important role in the European cosmetic market. The cosmetic industry in Italy was estimated to be about EUR 9 billion in 2007 (Wikipedia, 2012).

1.2.6 Economical potential of new cosmetic products

Decisions to buy and use cosmetics are related to different lifestyles and increasing consumer needs should be met by developing science and technology.

Innovation is significant because most cosmetic products have a lifetime of under five years and manufacturers reformulate a quarter of their products every year. Scientific research and development is important to the cosmetic industry. It can take several years to bring a product to the market and safety is built in at every stage in the process (Cosmetics Europe, 2012).

1.3 Future Perspective of Cosmetic Products

1.3.1 Petrochemical ingredients of cosmetic products

Petrochemicals are chemical products made from the raw materials of petroleum or other hydrocarbons. Olefins (including ethylene and propylene) and aromatics (including benzene and xylene isomers) are two main classes of petrochemicals (Fabsugar, 2012).

Petrochemicals are used in beauty products and toiletries, in the form of compounds such as propylene glycol and benzene, and are also used in the automotive and aviation industries, in explosives, plastics, soaps and detergents, dyes, food packaging, pharmaceutical preparations, home furnishings, carpet backing, refrigerators, moulded products, technology such as microchips in computers and CDs, synthetic fibres for clothes and furniture, rubbers, paints, bath and shower doors, paint thinner, toys, diaper covers, textiles, anti-freeze, floor polish, pens and pencils, lighters, dinnerware, insulation materials, and chemical intermediates. In addition, petrochemicals are used to treat non stick frying pans and for many more applications (Toxic Beauty, 2009).

According to recent research, petrochemical compounds used in cosmetics have adverse health effects. For example, one of the most common petrochemicals is petrolatum. Petrolatum, which is one of many petroleum-based products, is used in lipsticks and other cosmetics. Petrolatum can contain impurities linked to cancer and other health issues (Toxic Beauty, 2009).

1.3.2 Health risks of cosmetic products

Cosmetics include a wide range of products. Some of these can cause health problems in some people, including skin or eye irritations or allergic reactions when the product is used. On the other hand, whether cosmetics or their ingredients can cause more long-term health problems is still a matter of debate. Another important point for human health is based on the currently available data, which suggests that some cosmetic products increase cancer risks (American Cancer Society, 2012).

The skin of humans is extremely permeable, so cosmetic ingredients are absorbed through the skin. Some chemicals may penetrate the skin in significant amounts, especially when left on the skin for long periods, as in the case of facial makeup (Cancer Prevention Coalition, 2012).

1.3.3. Application potential of hazardous chemical free cosmetic products

There are hundreds of toxic ingredients in cosmetic products today that have not been tested for safety. They cannot only harm or irritate your delicate facial skin, but can also seriously affect human health in the long-term.

Today, natural production is an alternative to chemicals, and "clean chemistry" can develop new ingredients derived from natural and renewable resources, while respecting both biodiversity and sustainable development relating to increasing interest in both natural and bio-based ingredients (Cancer Prevention Coalition, 2012).

Synthetic emollients cause skin irritation, whilst natural emollients nourish the skin. Natural emollients are metabolised by the skin's own enzymes and absorbed into it, and are readily biodegradable. Some natural emollients are plant oils, and shea, cocoa and jojoba butters.

Natural phospholipids from lecithin are fantastic humectants. Phospholipids increase the hydration levels of the skin without being occlusive. Topically-applied plant phospholipids restore the barrier function of the skin, protecting it from substances such as bacteria and harmful chemicals. Some natural humectants are lecithin, penthanol and glycerine.

Synthetic emulsifiers are usually petroleum / hydrocarbon derivates and can be allergens. Natural emulsifiers are obtained from various nuts, berries and leaves. Some natural emulsifiers are plant waxes, xanthan gum and quince seed.

A serious problem with ethoxylated surfactants is that they can be contaminated with dioxane, a potent carcinogen. Natural saponins (foaming agents) are a much better choice for shampoos. They gently cleanse the hair and scalp without stripping the natural oils. Some natural surfactants include castile soap, yucca extract, soapwort and quillaja bark extract.

Chemical preservatives are usually used, because they are much cheaper and extend the shelf life of the product in comparison to natural alternatives. Storing natural products in the fridge will help to extend their life. Some natural preservatives include tea tree essential oil, thyme essential oil, grapefruit seed extract, and D-Alpha tocopherol acetate (Inspiredliving.com, 2012).

1.3.4. Research and development of new cosmetic products

Cosmetic science has a long history of discovery and innovation from areas including biology, chemistry, and physics. For years, scientists dealing with both fundamental and applied research have brought innovative applications to the

cosmetic community. Cosmetic product development is needed to stay up to date with the major changes in the cosmetics industry (The Centre for Professional Advancement, 2012).

Today, aerosol technology, emulsion-suspension technology, nanotechnology, granulation, microencapsulation, crystallisation technology, industrial technology, powder mixing technology, and industrial drying are used to develop products in cosmetics (The Centre for Professional Advancement, 2012).

1.4 Main Goals of This Study

Water which has a considerably high level of ^{18}O is evaporated more slowly with respect to regular water, resulting in keeping the skin more hydrated, which means clearer, healthier, and more brilliant skin. This also means that ^{18}O-enriched water may be used in the preparation of healthy cosmetic products.

The aim of this study is the investigation of the moisturising effect of skin moisturisers that are prepared by using ^{18}O-enriched water instead of light water.

2. EXPERIMENTAL

2.1 Specifications of Materials and Equipment

2.1.1 Purchasing the moisturiser which was used for studies

Moisturisers can help to increase the hydration of the human skin and can repair the protective barrier via their occlusive and humectant agents. These ingredients are the same or similar to natural components in the skin. Occlusive agents block the loss of water from the skin, whilst humectant agents attract water to the skin (Marino, 2001).

''Most moisturisers contain 65 – 85 % water in a lotion form with water acting as an agent to disperse the active and inactive ingredients. The high water content also serves to allow the absorption of some components and the evaporation of the moisturiser. The water acts as a temporary hydrating agent. Moisturiser in a cream form contains less water and more oil (Marino, 2001).''

A Vaseline brand moisturiser was chosen from the market for using in this study.

Figure 2.1.1 The moisturiser which was used in the experiments.

2.1.2 Purchasing of natural water which was added in moisturiser

The natural pure water which was used in this study, was purchased from a water purification company (Çiçekçi Ticaret, Izmir). The analytical characteristics of this water are given in Table 2.1.2.

Table 2.1.2 Cationic and anionic ingredients of pure water used in this study for evaporations.

Cationic and anionic ingredients	Measurement method	Measured values (mg / L)
Copper (Cu)	ICP-MS	0.003
Zinc (Zn)	ICP-MS	0.005
Iron (Fe)	ICP-MS	5.000
Fluoride (F⁻)	IC	0.030
Calcium (Ca)	IC	1.000
Chloride (Cl⁻)	IC	1.000
Lead (Pb)	ICP-MS	2.000
Magnesium (Mg)	IC	1.000
Nitrate (NO_3^-)	IC	1.000
Potassium (K)	IC	0.300
Sodium (Na)	IC	0.800
Total Hardness	Titrimetry	0.200
Nitrite (NO_2)	IC	0.100
Ammonia (NH_3)	IC	0.200

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As shown in Table 2.1.2, water used for enrichment processes in this study was natural pure water and its ^{18}O natural ratio was measured to be 0.19874 $\pm$ 0.00003% by Iso-Analytical Ltd. in the UK.

2.1.3 Purchasing of ^{18}O enriched water

$H_2^{18}O$ with an enrichment ratio of 97 % was purchased from the Shangai Research Institute of Chemical Industry in China for this study. In order to test the reliability of ^{18}O measurements carried out by Iso-Analytical Ltd. in the UK, the analytical characteristics of $H_2^{18}O$ are given in Tables 2.1.3.1 and 2.1.3.2.

Table 2.1.3.1 Analytical characteristics of 97 % enriched $H_2{}^{18}O$.

Isotopic Analyses	Units	Measured Values
^{18}O	Atom %	97.5
^{17}O	Atom %	0.7
^{16}O	Atom %	1.8
Chemical Purity		99.99 % +
Electrical conductivity	μS / cm	0.52
Pyrogen	EU / mL	0.12
Sterility		Passed
pH		7.0
Total organic carbon	mg / L	0.2
Fluoride (F)	mg / L	0.002
Chloride (Cl)	mg / L	0.002
Bromide (Br)	mg / L	0.002
Iodide (I)	mg / L	0.002
Calcium (Ca)	mg / L	0.02
Magnesium (Mh)	mg / L	0.02
Sodium (Na)	mg / L	0.2
Potassium (K)	mg / L	0.02
Copper (Cu)	mg / L	0.01
Iron (Fe)	mg / L	0.02
Phosphate	mg / L	0.01
Nitrate	mg / L	0,01

Table 2.1.3.2 Analytical characteristics of 10 % enriched $H_2{}^{18}O$.

Isotopic Analyses	Units	Measured Values
^{18}O	Atom %	10.5
^{17}O	Atom %	0.06
^{16}O	Atom %	89.44
Chemical Purity		99.99 % +
Electrical conductivity	μS / cm	1.2
pH		6.9
Total organic carbon	mg / L	0.3
Fluoride (F)	mg / L	0.002

Chloride (Cl)	mg / L	0.002
Bromide (Br)	mg / L	0.002
Iodide (I)	mg / L	0.002
Calcium (Ca)	mg / L	0.03
Magnesium (Mh)	mg / L	0.01
Sodium (Na)	mg / L	0.1
Potassium (K)	mg / L	0.02
Copper (Cu)	mg / L	0.02
Iron (Fe)	mg / L	0.02
Phosphate	mg / L	0.01
Nitrate	mg / L	0,01

2.1.4 Digital moisture monitor for skin measurements

In order to determine the moisture content of the skin, a digital moisture monitor was used. The technical specifications of this monitor are as follows: Operating temperature: 5 – 40 °C; Operating relative humidity: < 70 %; Measurable moisture range: 0 - 99.9 %; Dimension: 131 mm x 27 mm x 24 mm; Net weight: 40 g.

Figure 2.1.4 A view of digital moisture monitor which was used in this study for measurements of skin.

2.1.5 Hot-plate used for water evaporation

The technical specifications of the hot-plate used for water evaporation of the moisturisers are as follows: Mark: Esco; Type: EHP401; 230 V, 50 Hz; Power: 1000 W; Current: 4.3 A.

Figure 2.1.5 The hot – plate which was used in the experiments.

2.1.6 Balance

In order to determine the evaporated quantities of water and for the addition of water a balance was used. The technical specifications of this balance are as follows: DESIS marked; THB – 600 model; d = 0.01 g.

Figure 2.1.6 The balance which was used in the experiments.

2.2 Evaporation of the Moisturiser

2.2.1 Evaporation in silicon oil bath

The area of the digital moisture monitor was effectively 0.63 cm^2, but since it is difficult to assess, the measurement area was determined as 1.26 cm^2. After that, the amount of moisturiser was determined for this area (0.01 g) and 5 g moisturiser was put in an aluminium foil for evaporation. The evaporation was carried out at 41°C in silicon oil bath. The temperature was chosen as 41°C, because the other materials in the moisturiser should not be evaporated. Thus, the structure of moisturiser was not affected.

Figure 2.2 The evaporation operation set up.

After the start of evaporation process, the moisturiser was weighed periodically. At the end, total evaporation was determined to be 65.2 % of the total weight of moisturiser. The results are shown in Table 2.2.1.

Table 2.2.1 The moisturiser mass of evaporation in silicon oil bath.

Time (hour)	Mass (g) (moisturiser)	Weight Loss After Evaporation (g)	The Percentage of Evaporation
0	5.00	-	-
70	2.23	2.77	55.4 %
73	2.18	2.82	56.4 %
76	2.16	2.84	56.8 %
93	2.04	2.96	59.2 %
95	2.03	2.97	59.4 %

97	2.00	3.00	60.0 %
99	2.00	3.00	60.0 %
117	1.92	3.08	61.6 %
120	1.90	3.10	62.0 %
122	1.90	3.10	62.0 %
124	1.87	3.13	62.6 %
165	1.74	3.26	**65.2 %**
168	1.74	3.26	**65.2 %**
170	1.74	3.26	**65.2 %**
172	1.74	3.26	**65.2 %**

2.2.2 Evaporation at room temperature

Without any heating effect, 5 g of moisturiser was left for the evaporation at room temperature, in order to observe the percentage of water that will be lost. Total evaporation was also determined to be 65.2 % of the total weight of moisturiser. The results are shown in Table 2.2.2.

Table 2.2.2 The moisturiser mass evaporated at room temperature.

Time (Week)	Mass (g) (Moisturiser)	Weight Loss After Evaporation (g)	The Percentage of Evaporation
1	2.46	2.54	50.80 %
3	2.07	2.93	58.60 %
4	1.88	3.12	62.40 %
5	1.78	3.22	64.40 %
6	1.78	3.22	64.40 %
8	1.74	3.26	**65.20 %**
10	1.74	3.26	**65.20 %**
12	1.74	3.26	**65.20 %**

2.3 Preparation of ^{18}O Enriched Water with different Enrichment Levels

2.3.1 ^{18}O Enriched Water with 1 % Enrichment Level

^{18}O enriched water with 1 % enrichment level was prepared using ^{18}O enriched water with 10 % enrichment level which was purchased from Chinese company. For this, 15.56 µL of 10 % enriched water was mixed with 184.44 µL pure water according to the following calculations:

$$(\%_1 \text{ x } V_1) + (\%_2 \text{ x } V_2) = \% \text{ x } V$$

$$(10.5 \text{ x } V_1) + (0.198 \text{ x } (200 - V_1)) = 1 \text{ x } 200$$

$$10.5 \, V_1 + 39.6 - 0.198 \, V_1 = 200$$

$$10.232 \, V_1 = 160.4 \quad \longrightarrow \quad V_1 = 15.56 \, \mu L; \, V_2 = 184.44 \, \mu L$$

Thus, 200 µL of ^{18}O enriched water with 1 % enrichment level was obtained. But, the exact ^{18}O enrichment level of a sample taken from this water was measured by Iso-Analytical Ltd. in the UK to be 0.87976 % ($\pm$ 0.00003).

2.3.2 ^{18}O Enriched Water with 50 % Enrichment Level

^{18}O enriched water with 50 % enrichment level was prepared using ^{18}O enriched water with 97 % enrichment level which was purchased from Chinese company. For this, 102.36 µL of 97 % enriched water was mixed with 97.63 µL pure water according to the following calculations:

$$(\%_1 \text{ x } V_1) + (\%_2 \text{ x } V_2) = \% \text{ x } V$$

$$(97.5 \text{ x } V_1) + (0.198 \text{ x } (200 - V_1)) = 50 \text{ x } 200$$

$$97.5 \, V_1 + 39.6 - 0.198 \, V_1 = 10000$$

$$97.302 \, V_1 = 9960.4 \quad \longrightarrow \quad V_1 = 102.36 \, \mu L; \, V_2 = 97.63 \, \mu L$$

Thus, 200 µL of ^{18}O enriched water with 50 % enrichment level was obtained. But, the exact ^{18}O enrichment level of a sample taken from this water was measured by Iso-Analytical Ltd. in the UK to be 48.79762 % ($\pm$ 0.00003).

2.4 Preparation of New Moisturisers

2.4.1 Preparation of new moisturisers with light water

The amount of dry moisturiser which was remained after evaporation, was 1.74 g and 0.5 g was taken from the dry moisturiser for preparation of new moisturisers:

1.74 g Dry moisturiser → 3.26 g Light water (Total = 5 g)

0.5 g Dry moisturiser → 0.93 g Light water (Total = 1.43 g)

0.93 g Light water was added to 0.5 g of dry moisturiser.

This new moisturiser will be mentioned in the following paragraphs and sections with the abbreviation MPLW (Moisturiser Prepared with Light Water).

MPLWs were prepared from the dry moisturiser that was remained after evaporation. Three different MPLWs were prepared; they were consisted of 32.81 %, 65.2 %, and 80 % of pure water, respectively. The quantities were calculated to contain 0.005 g of dry moisturiser for each sample. The quantities of pure water which was added to dry moisturiser, are given in the Table 2.4.1.

Table 2.4.1 The quantity of moisturiser which should be consisted of 0.005 g of dry moisturiser for each sample.

Quantity	Dry moisturiser (g)	Total (g)
32.81 % Light Water	67.19	100
	0.005	0.0074
65.2 % Light Water	34.80	100
	0.005	0.0143
80 % Light Water	20.00	100
	0.005	0.025

2.4.2 Preparation of new moisturisers with ^{18}O enriched water

Similarly, the other new moisturisers were prepared with ^{18}O enriched water, having different ^{18}O enrichment levels. At first, 0.07 g of the dry

moisturiser was taken for each new moisturiser. Then, 0.13 g of ^{18}O enriched water with different ^{18}O enrichment levels to be 1 %, 10 %, and 50 % was separately added to this dry moisturiser.

2.5 Moisturizing Measurements of Skin

Two different areas were chosen for moisturizing measurements of skin; these were hand and arm as shown in Figures 2.5.1, 2.

Three equal areas were principally determined on hand and arm with about 1.26 cm$^{2\,of}$ each for these measurements. The following procedures were applied:

1. 0.01g of Commercially-available moisturiser was applied on the skin.

2. 0.01g of Moisturiser with light water was applied on the skin.

3. Skin was not treated with moisturiser.

The moisture contents of the skin at these squares were then measured after the specified time intervals. The same procedures were repeated for the other three squares determined on the arm.

At the second step of these measurements, four similar squares at the same areas of hand and arm. The following procedures were applied separately for hand and arm:

1. 0.01g of Commercially-available moisturiser was applied on the skin.

2. 0.01g of Moisturiser with pure water was applied on the skin.

3. 0.01g of Dry moisturiser (water was evaporated) was applied on the skin.

4. Skin was not treated with moisturiser.

The moisture contents of the skin at these squares on hand and arm were then separately measured after the specified time intervals.

Particularly, the calculated amounts of moisturisers were applied on three different squares of the hand (Table 2.4.2). 0.005 g Dry moisturiser was applied on the fourth area and no moisturiser was applied to the fifth area. The moisture content was then measured at the specified time intervals at each of the four squares. The same procedures were repeated in the other five squares which were selected in the arm.

At another step of these measurements, six equal areas were determined on hand and arm for measurements ($1.26cm^2$). The following procedures were applied:

1. 0.01g of Commercially-available moisturiser was applied on the skin.

2. 0.01g of Moisturiser with pure water was applied on the skin.

3. 0.01g of Moisturiser with ^{18}O enriched water with 1 % enrichment level was applied on the skin.

4. 0.01g of Moisturiser with ^{18}O enriched water with 10 % enrichment level was applied on the skin.

5. 0.01g of Moisturiser with ^{18}O enriched water with 50 % enrichment level was applied on the skin.

6. Skin was not treated with moisturiser.

The moisture content was then separately measured for each of the specified time intervals at the six squares on hand and arm.

Figure 2.5.1 The squares of measurements on hand and arm areas.

For all measurements, the moisturisers were applied on the skin using a tiny spatula and the measurement results were obtained using a digital moisture measurement device. Each measurement was taken about 10 seconds.

Figure 2.5.2 Moisturizing measurements of skin.

2.6 Cell Culture Experiments

The cell culture studies were carried out at the Izmir Institute of Technology to investigate the toxic effects of [18]O enriched water on live skin cells.

The NIH3T3 (ATCC mouse embryonic fibroblast-) cell line was used for this study. Cell lines were maintained in an incubator at 37 °C which contains 5 % CO_2. During the reproduction of the cells, DMEM media (Gibco), which contained Penicillin (100 U / mL) (Gibco), streptomycin (100 mcg / mL) (Gibco), 10 % FBS (Gibco), and 4.5 g / L % glucose and 2 mM L-Glutamine, was used.

DMEM media in dust form was dissolved in 1.5 mL of pure light water and in the same volume of [18]O enriched water with different enrichment level (1 %, 10 %, and 50 %). Dust media which allowed the preparation of 5 mL of media was weighed in order to be proportional to the volume of 1.5 mL. The media was sterilised using 0.22 μm syringe filters in a Class II biosafety cabinet.

As a part of the experiment, the cell population treated with media which was prepared using pure light water was considered as a negative control. Before this step, fibroblast cells in 96-well plates, in a volume of 100 mL and cultivated for 24 hours, were calculated to be at a density of 10^4 cells, and an adsorption

curve was obtained for evaluation of incubation results. The experiment was performed in triplicate for each sample.

The cells were inoculated with liquid DMEM media during reproduction. At this stage, penicillin and streptomycin were not added to the media to prevent any effect on the test results.

After 24 hours, the media was removed and a different ^{18}O enriched water, for example with 1 % enrichment level, was added. The cells were incubated for 24 hours with 5 % CO_2 at 37°C before analysis. After 24 hours, 1 x PBS (Phosphate Buffer Saline) in the MTT (3-{4,5-dimethylthiazol-2-yl}-2,5-diphenyl tetrazolium bromide) was prepared by dissolving 5 mg / mL of stock solution to determine the cytotoxic activity. The stock solution was diluted to a working concentration of 0.5 mg / mL for analysis in order to maintain its chemical stability. After incubation, the 96 well plate was removed from the incubator and was inverted on a paper towel. Thus, the plate was impregnated by the paper and the prepared media was removed from the plate. For more effective removal of the residual media, wells were washed with 100 µL 1 x PBS / MTT, which was diluted to a working concentration and added into the wells. The plate was then incubated for 4 hours at 37°C. 100 µL of MTT solution was added into three empty wells to use as a blank in the spectrophotometric measurements.

After incubation, the plate was centrifuged at 1800 rpm for 10 minutes at room temperature to precipitate formazan crystals from the reaction. The supernatant was removed by impregnated paper whilst the formazan crystals were collected at the bottom of each well by adding 100 mL of DMSO, which was dissolved and stirred for 5 minutes. Different shades of a purple colour change were observed in wells. At the end of the period, a 545 nm wavelength spectrophotometric measurement was made using an ELISA Plate Reader. During the calculations, a replicate of each sample obtained for the average absorbance values was averaged and the blank value was removed from these values. Calculating the percentage of viability was performed using the following formula:

% Vitality = 100 × (Absorbance value of the sample mean / Negative control mean absorbance value)

3. RESULTS AND DISCUSSION

3.1 Results of Moisture Measurements

3.1.1 Comparison of the commercial moisturiser and the pure water-added moisturiser

The moisture measurements of the commercial moisturiser, the first evaporated sample and the same amount of pure water-added moisturiser show that only the water was evaporated from the moisturiser. The measurements of dry skin show that the moisture percent of the skin changes with environmental factors at the different time intervals. The results obtained were tabulated in the following Table 3.1.1.1.

Table 3.1.1.1 The measurements for different skin on hand and arm areas.

Time	HAND			ARM		
	Dry skin	Commercial moisturiser	Pure light water-added moisturiser	Dry skin	Commercial moisturiser	Pure light water-added moisturiser
(Hour)	(%)	(%)	(%)	(%)	(%)	(%)
0	30.96 ± 0.30	30.06 ± 0.23	31.20 ± 0.52	31.00 ± 0.10	31.33 ± 0.23	30.96 ± 0.30
0.25	31.86 ± 0.41	46.00 ± 0.30	45.23 ± 0.05	33.36 ± 0.45	50.06 ± 1.26	49.20 ± 0.10
1	30.16 ± 0.47	41.66 ± 0.52	41.20 ± 0.20	34.63 ± 0.23	43.73 ± 0.28	43.00 ± 0.10
3	31.36 ± 0.05	37.90 ± 0.34	37.66 ± 0.11	34.60 ± 0.17	41.10 ± 0.00	40.16 ± 0.20
6	29.53 ± 0.20	34.93 ± 0.50	36.16 ± 0.30	31.93 ± 0.15	37.60 ± 0.30	37.40 ± 0.17
12	31.26 ± 1.76	34.43 ± 0.41	34.00 ± 0.20	32.66 ± 0.05	36.30 ± 0.30	35.20 ± 0.17
24	30.00 ± 0.10	30.40 ± 0.34	30.40 ± 0.26	31.40 ± 0.40	32.00 ± 0.10	31.80 ± 0.26

The skin moisture value was subtracted from the moisture percent of the commercial and the pure water-added moisturisers separately. Thereby, only the percent of moisture given to the skin by the moisturisers were determined. Thus, the effect of each moisturiser over the skin moisture percent was investigated. The results obtained were tabulated in the following Table 3.1.1. 2 and are shown in Figures 3.1.1.1, 2.

Table 3.1.1.2 The moisture increasing on the skin of hand and arm.

Time (Hour)	HAND		Time (Hour)	ARM	
	Commercial moisturiser (%)	Pure light water-added moisturiser (%)		Commercial moisturiser (%)	Pure light water- added moisturiser (%)
0.25	14.14 ± 0.30	13.37 ± 0.05	0.25	16.70 ± 1.26	15.84 ± 0.10
1	11.50 ± 0.52	11.04 ± 0.20	1	9.10 ± 0.28	8.37 ± 0.10
3	6.54 ± 0.34	6.30 ± 0.11	3	6.50 ± 0.00	5.56 ± 0.20
6	5.40 ± 0.50	6.63 ± 0.30	6	5.67 ± 0.30	5.47 ± 0.17

| 12 | 3.17 ± 0.41 | 2.74 ± 0.20 | 12 | 3.64 ± 0.30 | 2.54 ± 0.17 |
| 24 | 0.40 ± 0.34 | 0.40 ± 0.26 | 24 | 0.60 ± 0.10 | 0.40 ± 0.26 |
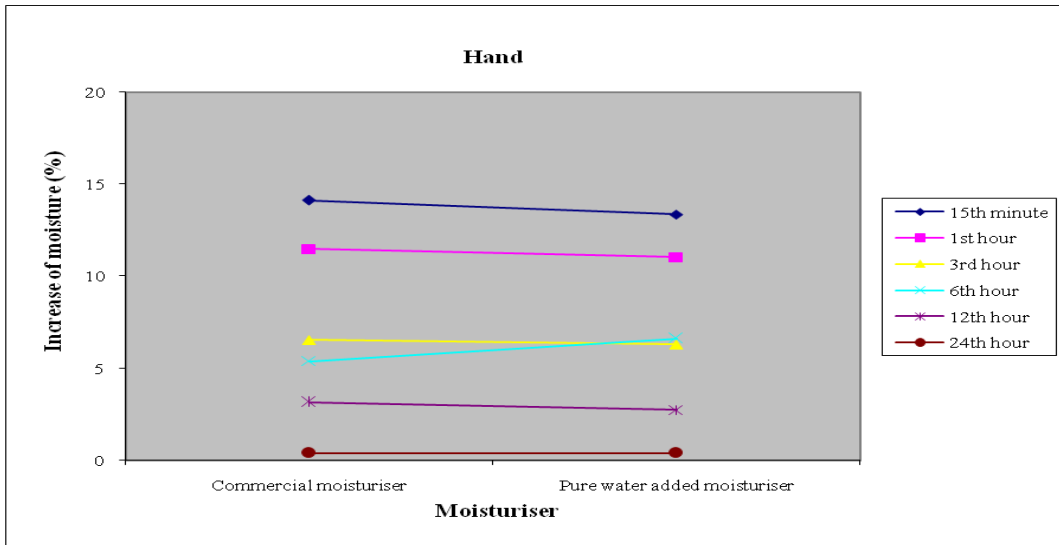
Figure 3.1.1.1 The moisture increasing on hand skin**.**
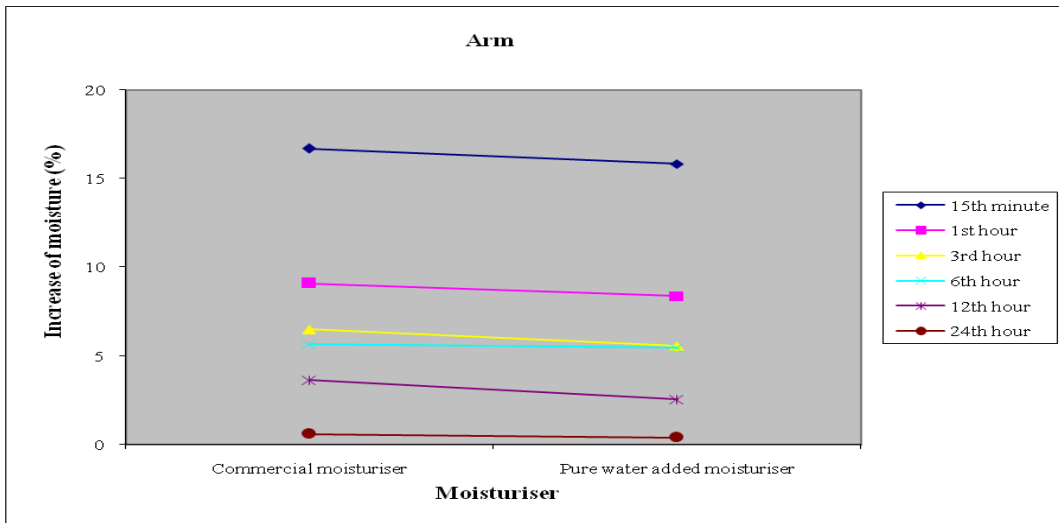
 Figure 3.1.1.2 The moisture increasing on arm skin .

3.1.2 Comparison of the commercial moisturiser, first evaporated and then the same amount of pure light water-added moisturiser as well as the dry moisturiser

The moisturising effects of the commercial moisturiser and the water-added moisturiser were similar and the moisturising effect of the dry moisturiser was higher, although the quantity of the moisturisers was the same (0.01 g). This is

because the quantity percent of the humectant and occlusive agents in the dry moisturiser is higher than in the other two moisturisers. The results obtained were tabulated in the following Table 3.1.2.1.

The skin moisture value was separately subtracted from the moisture percent of the commercial moisturiser, the pure light water-added moisturiser and dry moisturiser. Thereby, only the percent of moisture given to the skin by the moisturisers were determined. Thus, the effect of each moisturiser over the skin moisture percent was investigated. The results obtained were tabulated in the following Table 3.1.2.2 and are shown in Figures 3.1.2.1, 2.

Table 3.1.2.1 The measurements of skin on hand and arm.

HAND				
Time (Hour)	Dry skin (%)	Dry moisturiser (%)	Commercial moisturiser (%)	Pure light water-added moisturiser (%)
0	28.93 ± 0.15	29.86 ± 1.35	30.40 ± 0.36	30.00 ± 0.10
0.25	29.43 ± 0.50	50.86 ± 2.59	50.36 ± 0.41	50.43 ± 0.50
1	31.60 ± 0.10	49.56 ± 0.47	48.00 ± 0.36	48.10 ± 0.20
3	32.30 ± 0.72	48.53 ± 0.20	46.40 ± 0.26	47.83 ± 0.11
6	32.80 ± 0.51	45.20 ± 0.34	41.20 ± 0.60	41.33 ± 0.05
12	29.96 ± 0.35	40.63 ± 0.25	39.63 ± 0.40	39.96 ± 0.23
24	29.10 ± 0.17	34.13 ± 0.35	35.10 ± 0.85	34.80 ± 0.17
ARM				
Time (Hour)	Dry skin (%)	Dry moisturiser (%)	Commercial moisturiser (%)	Pure light water-added moisturiser (%)
0	30.56 ± 0.05	30.70 ± 0.26	31.06 ± 0.11	30.86 ± 0.20
0.25	29.93 ± 0.20	46.96 ± 0.51	46.00 ± 0.70	46.80 ± 0.26
1	32.30 ± 0.43	46.80 ± 0.51	45.70 ± 0.00	44.76 ± 0.15
3	34.03 ± 0.11	45.36 ± 0.20	44.93 ± 0.25	43.63 ± 0.37
6	32.26 ± 0.28	44.63 ± 0.23	42.70 ± 0.40	41.20 ± 0.17
12	30.83 ± 0.40	45.60 ± 0.20	40.86 ± 0.25	40.50 ± 0.43
24	29.50 ± 0.10	36.03 ± 1.15	33.80 ± 0.17	34.20 ± 0.40

Table 3.1.2.2 The moisture increasing on skin of hand and arm.

Time (Hour)	HAND			ARM		
	Dry moisturiser (%)	Commercial moisturiser (%)	Pure light water-added moisturiser (%)	Dry moisturiser (%)	Commercial moisturiser (%)	Pure light water-added moisturiser (%)
0.25	21.43 ± 1.35	20.93 ± 0.41	21.00 ± 0.50	17.03 ± 0.51	16.07 ± 0.70	16.87 ± 0.26
1	18.56 ± 2.59	16.40 ± 0.36	16.50 ± 0.20	14.50 ± 0.51	13.40 ± 0.00	12.46 ± 0.15
3	16.23 ± 0.47	14.10 ± 0.26	15.53 ± 0.11	11.33 ± 0.20	10.90 ± 0.25	9.60 ± 0.37
6	12.40 ± 0.20	8.40 ± 0.60	8.53 ± 0.05	12.37 ± 0.23	10.44 ± 0.40	8.94 ± 0.17
12	10.67 ± 0.34	9.67 ± 0.40	10.00 ± 0.23	14.77 ± 0.20	10.03 ± 0.25	9.67 ± 0.43
24	5.03 ± 0.25	6.00 ± 0.85	5.70 ± 0.17	6.53 ± 1.15	4.30 ± 0.17	4.70 ± 0.40
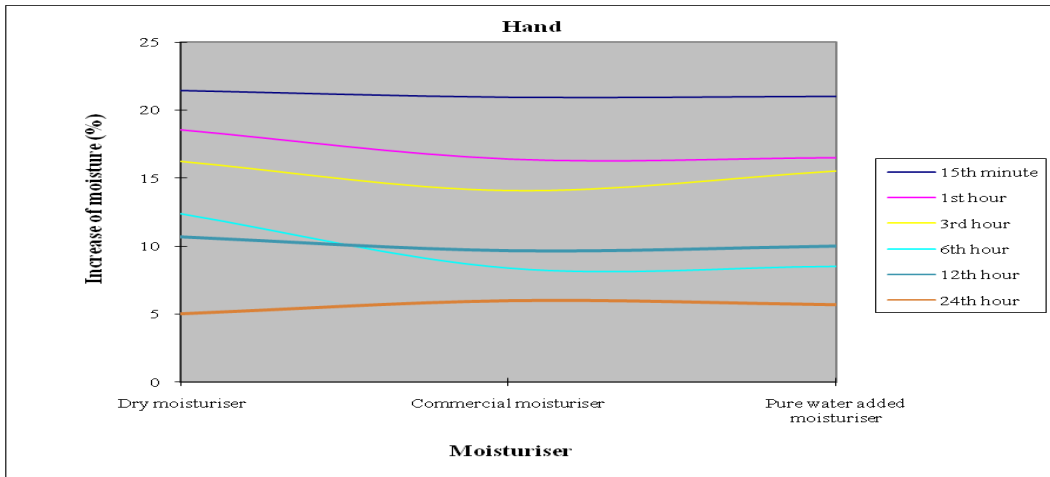
Figure 3.1.2.1 The moisture increasing on hand skin.
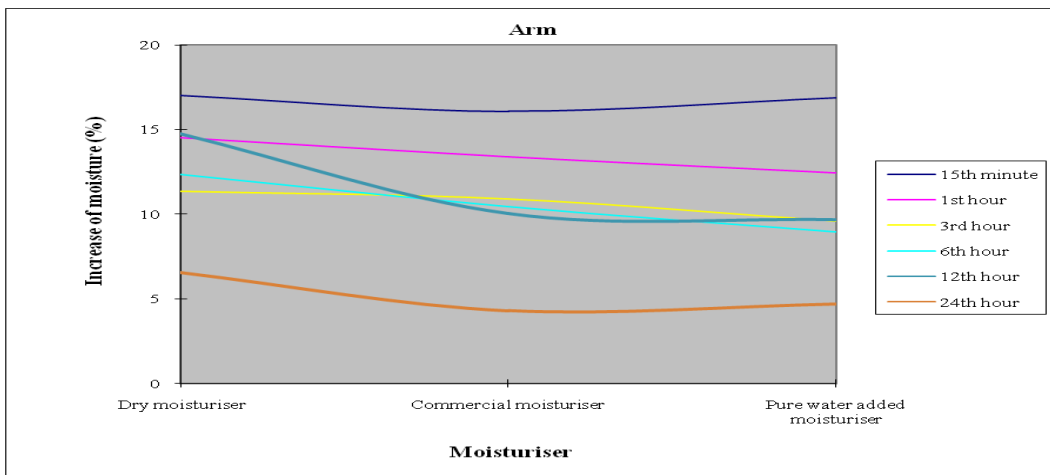
Figure 3.1.2.2 The moisture increasing on arm skin.

3.1.3 Investigation of the effect of water percent in the moisturiser on the skin moisture percent

Since all of the moisturiser samples include the same amount of dry moisturiser, this meant that the amounts of hygroscopic and desiccant agents were constant for all the moisturisers. Thus, only the water effect on the skin moisture percent was investigated. The moisture percent of the commercial moisturiser, first evaporated moisturiser, and the same amount of pure light water-added moisturiser (65.2 %) were similarly higher than the other moisturisers. The 32.81 % water moisturiser had the second highest moisture percent for either arm or hand skins. The dry moisturiser followed these moisturisers and the 80 % water moisturiser had the lowest moisture results on arm skin whilst the dry moisturiser had the lowest moisture results on hand skin. This result illustrated the fact that

the increase in the amount of water exceeded the absorption capacity of the skin. Thus, all of the sample cannot be absorbed into the skin. Because of this, the amount of humectant and occlusive agents absorbed by the skin is lower than the other areas. The results obtained were tabulated in the following tables, Table 3.1.3.1, 2 and are shown in Figures 3.1.2.1, 2.

Table 3.1.3.1 The measurements for different skin areas on hand and arm.

Time (Hour)	Dry skin (%)	Dry moisturiser (%)	32.81 % Pure light water-added moisturiser (%)	65.2 % Pure light water-added moisturiser (%)	Commercial moisturiser (%)	80 % Pure light water-added moisturiser (%)
HAND						
0	29.53 ± 0.55	29.16 ± 0.56	29.30 ± 0.30	30.00 ± 0.32	30.96 ± 1.68	29.80 ± 0.86
0.25	30.80 ± 0.60	37.00 ± 2.13	41.26 ± 1.06	43.80 ± 1.64	43.03 ± 0.86	43.03 ± 0.46
1	35.80 ± 0.62	43.90 ± 1.15	43.80 ± 0.75	50.73 ± 0.70	48.90 ± 0.40	45.76 ± 0.63
3	35.46 ± 0.89	40.10 ± 0.10	39.06 ± 0.68	44.10 ± 1.10	44.30 ± 0.98	40.20 ± 0.60
6	31.16 ± 0.94	35.90 ± 0.10	36.70 ± 0.75	40.03 ± 0.66	40.00 ± 1.12	36.66 ± 0.51
12	34.46 ± 0.85	38.10 ± 0.10	38.16 ±1.30	40.56 ± 0.95	40.13 ± 1.47	37.06 ± 0.68
24	30.63 ± 1.55	35.30 ± 0.36	32.26 ± 0.64	39.23 ± 1.33	38.96 ± 0.98	35.93 ± 1.40
ARM						
0	29.53 ± 0.30	30.03 ± 0.11	29.43 ± 0.28	28.93 ± 0.23	29.16 ± 0.05	29.40 ± 0.10
0.25	29.30 ± 0.40	39.70 ± 2.00	40.56 ± 2.27	42.76 ± 2.53	42.10 ± 0.55	37.63 ± 0.47
1	29.40 ± 0.36	42.00 ± 0.36	42.00 ± 0.43	43.96 ± 0.30	41.06 ± 0.37	36.90 ± 0.43
3	29.76 ± 0.11	39.73 ± 0.15	41.93 ± 0.75	42.33 ± 0.07	40.36 ± 0.47	37.66 ± 0.46
6	30.36 ± 0.05	37.90 ± 0.10	38.63 ± 0.75	39.63 ± 0.07	39.70 ± 0.26	35.30 ± 0.36
12	29.96 ± 0.40	38.63 ± 0.95	41.70 ± 0.52	38.63 ± 0.56	38.86 ± 0.41	38.76 ± 0.51
24	29.46 ± 0.35	35.50 ± 0.17	37.33 ± 0.83	38.60 ± 0.34	36.23 ± 0.32	34.90 ± 0.36

Table 3.1.3.2 The moisture increasing on skin of hand and arm.

Time (Hour)	Dry moisturiser (%)	32.81 % Pure light water- added moisturiser (%)	65.2 % Pure light water added moisturiser (%)	Commercial moisturiser (%)	80 % Pure light water added moisturiser (%)
HAND					
0.25	6.20 ± 2.13	10.46 ± 1.06	13.00 ± 1.64	12.23 ± 0.86	12.23 ± 0.46
1	8.10 ± 1.15	8.00 ± 0.75	14.93 ± 0.70	13.10 ± 0.40	9.96 ± 0.63
3	4.64 ± 0.10	3.60 ± 0.68	8.64 ± 1.10	8.84 ± 0.98	4.74 ± 0.60
6	4.74 ± 0.10	5.54 ± 0.75	8.87 ± 0.66	8.84 ± 1.12	5.50 ± 0.51
12	3.64 ± 0.10	3.70 ± 1.30	6.10 ± 0.95	5.67 ± 1.47	2.60 ± 0.68
24	4.67 ±0.36	1.63 ± 0.64	8.60 ± 1.33	8.33 ± 0.98	5.30 ± 1.40
ARM					
0.25	10.40 ± 2.00	11.26 ± 2.27	13.46 ± 2.53	12.80 ± 0.55	8.33 ± 0.47
1	12.60 ± 0.36	12.60 ± 0.43	14.56 ± 0.30	11.66 ± 0.37	7.50 ± 0.43
3	9.97 ± 0.15	12.17 ± 0.75	12.57 ± 0.07	10.60 ± 0.47	7.90 ± 0.46
6	7.54 ± 0.10	8.27 ± 0.75	9.27 ± 0.07	9.34 ± 0.26	4.94 ± 0.36
12	8.67 ± 0.95	11.74 ± 0.52	8.67 ± 0.56	8.90 ± 0.41	8.80 ± 0.51
24	6.04 ± 0.17	7.87 ± 0.83	9.14 ± 0.34	6.77 ± 0.32	5.44 ± 0.36
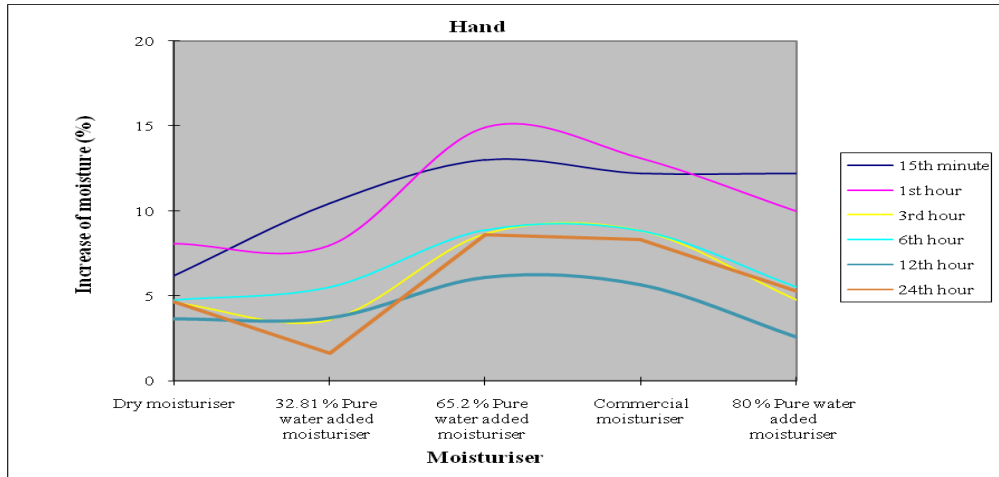
Figure 3.1.3.1 The moisture increasing on hand skin.
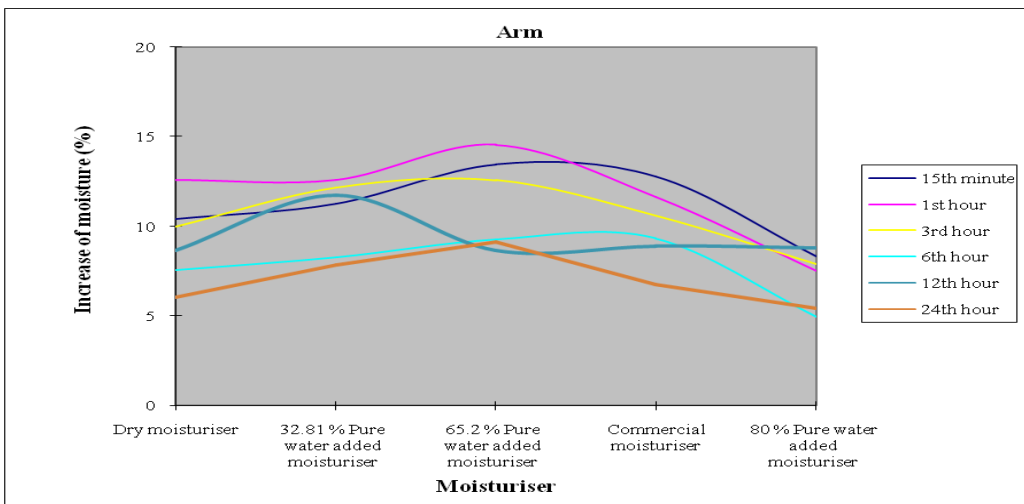
Figure 3.1.3.2 The moisture increasing on arm skin.

3.1.4 Comparison of the commercial moisturiser, first evaporated and the same amount of ^{18}O enriched water-added moisturisers

The averages of the moisture percents of commercial and pure light water-added moisturisers were taken, and were considered as the values for the commercial moisturiser. The skin moisture value was separately subtracted from the moisture percent of the commercial and the ^{18}O enriched water-added moisturisers. Thereby, only the percent of moisture given to the skin by the moisturisers were determined. Thus, the effect of each moisturiser over the skin moisture percent was investigated. The results obtained were tabulated in the following tables, Table 3.1.4.1, 2 and are shown in Figures 3.1.4.1, 2.

Table 3.1.4.1 The measurements of hand areas for different four people.

Person A – Hand					
Time (Hour)	Dry Skin (%)	Pure light water-added moisturiser (%)	Commercial moisturiser (%)	1 % ^{18}O enriched water- added moisturiser (%)	10 % ^{18}O enriched water- added moisturiser (%)	50 % ^{18}O enriched water- added moisturiser (%)
0	27.73 ± 0.66	27.66 ± 0.20	27.80 ± 0.20	27.56 ± 0.05	27.06 ± 0.11	27.96 ± 0.85
0.25	26.30 ± 0.26	35.86 ± 0.30	40.46 ± 0.75	36.83 ± 0.30	39.43 ± 0.15	41.36 ± 0.25
1	28.73 ± 0.11	35.06 ± 0.40	39.76 ± 0.05	35.93 ± 0.73	39.06 ± 0.61	39.33 ± 1.01
3	29.00 ± 1.08	33.00 ± 0.70	37.06 ± 0.50	34.53 ± 0.25	37.36 ± 0.80	37.46 ± 0.47
6	29.00 ± 1.90	33.56 ± 0.85	37.50 ± 1.32	35.43 ± 0.60	39.10 ± 0.72	38.36 ± 1.32
12	29.63 ± 0.32	28.90 ± 0.10	32.80 ± 0.91	31.90 ± 0.17	32.73 ± 2.29	37.30 ± 0.20
24	30.96 ± 0.30	31.16 ± 1.16	34.20 ± 0.70	32.60 ± 0.85	35.66 ± 0.73	35.00 ± 1.83
Person B – Hand					
Time (Hour)	Dry Skin (%)	Pure light water-added moisturiser (%)	Commercial moisturiser (%)	1 % ^{18}O enriched water- added moisturiser (%)	10 % ^{18}O enriched water- added moisturiser (%)	50 % ^{18}O enriched water- added moisturiser (%)
0	22.43 ± 0.90	22.80 ± 0.10	22.83 ± 0.81	22. 90 ± 0.72	22.33 ± 0.32	22.33 ± 1.09
0.25	22.90 ± 1.15	44.20 ± 0.20	44.00 ± 2.75	42.80 ± 1.99	51.26 ± 1.12	56.86 ± 0.45
1	25.93 ± 1.35	40.10 ± 0.17	39.20 ± 1.22	39.60 ± 0.79	46.33 ± 1.84	53.16 ± 0.80
3	30.13 ± 0.83	44.30 ± 0.17	45.23 ± 0.90	44.63 ± 0.80	49.56 ± 0.47	54.83 ± 0.97
6	30.93 ± 1.10	35.76 ± 1.06	38.80 ± 0.66	38.80 ± 0.10	45.60 ± 1.51	40.20 ± 0.20
12	31.70 ± 1.00	35.30 ± 0.10	35.50 ± 0.30	36.76± 1.35	36.63 ± 0.58	36.13 ± 0.56
24	28.70 ± 0.50	29.30 ± 0.65	30.10 ± 0.20	30.33 ± 0.92	29.66 ± 0.15	30.33 ± 0.15
Person C – Hand					
Time (Hour)	Dry Skin (%)	Pure light water-added moisturiser (%)	Commercial moisturiser (%)	1 % ^{18}O enriched water- added moisturiser (%)	10 % ^{18}O enriched water- added moisturiser (%)	50 % ^{18}O enriched water- added moisturiser (%)
0	29.10 ± 0.26	29.10 ± 0.45	29.50 ± 0.10	29.86 ± 0.58	29.40 ± 0.20	29.60 ± 0.40
0.25	29.40 ± 0.65	50.23 ± 0.77	50.76 ± 0.77	55.53 ± 0.90	55.33 ± 0.37	58.90 ± 1.24
1	31.93 ± 0.51	48.23 ± 1.01	49.10 ± 0.62	52.36 ± 0.73	50.80 ± 0.70	57.00 ± 0.92
3	29.26 ± 0.83	45.76 ± 0.66	44.46 ± 0.32	52.46 ± 0.37	49.50 ± 0.51	56.13 ± 0.28
6	30.83 ± 0.40	42.86 ± 0.49	43.06 ± 0.25	49.00 ± 0.72	49.53 ± 0.76	52.86 ± 0.55
12	30.46 ± 0.75	39.46 ± 0.73	39.23 ± 0.75	40.80 ± 0.90	40.23 ± 1.24	49.30 ± 0.88
24	30.00 ± 0.10	32.10 ± 0.10	32.30 ± 0.30	34.90 ± 0.10	35.20 ± 0.20	36.20 ± 0.20
Person D – Hand					
Time (Hour)	Dry Skin (%)	Pure light water-added moisturiser (%)	Commercial moisturiser (%)	1 % ^{18}O enriched water- added moisturiser (%)	10 % ^{18}O enriched water- added moisturiser (%)	50 % ^{18}O enriched water- added moisturiser (%)
0	32.10 ± 0.26	31.73 ± 0.41	31.03 ± 0.75	32.20 ± 0.62	32.10 ± 0.10	32.00 ± 0.10
0.25	33.66 ± 0.73	48.66 ± 0.20	47.50 ± 0.20	51.26 ± 1.20	49.33 ± 0.68	53.80 ± 1.63
1	34.36 ± 0.95	46.13 ± 0.92	46.00 ± 0.30	46.96 ± 0.64	48.33 ± 0.80	51.76 ± 0.57
3	36.63 ± 0.55	43.76 ± 0.72	42.16 ± 1.34	45.43 ± 0.86	46.10 ± 1.50	50.60 ± 0.87
6	37.43 ± 0.72	43.80 ± 0.72	40.36 ± 0.73	46.86 ± 0.20	49.10 ± 0.78	50.43 ± 0.15
12	39.73 ± 0.60	40.10 ± 0.10	40.90 ± 1.00	40.20 ± 0.36	42.00 ± 0.20	40.90 ± 0.10
24	35.66 ± 0.11	37.80 ± 0.10	35.06 ± 0.90	42.00 ± 0.20	41.13 ± 0.58	42.40 ± 0.20

Table 3.1.4.2 The measurements of arm areas for different four people.

Person A – Arm					
Time (Hour)	Dry Skin (%)	Pure light water-added moisturiser (%)	Commercial moisturiser (%)	1 % ^{18}O enriched water- added moisturiser (%)	10 % ^{18}O enriched water- added moisturiser (%)	50 % ^{18}O enriched water- added moisturiser (%)
0	28.60 ± 0.55	28.56 ± 0.05	28.60 ± 0.43	28.53 ± 0.68	28.33 ± 0.51	29.16 ± 0.20
0.25	25.23 ±0.55	39.96 ± 0.25	40.16 ± 0.30	39.40 ± 0.70	41.10 ± 0.17	46.30 ± 0.62
1	24.73 ± 0.55	37.40 ± 0.36	38.06 ± 0.30	36.50 ± 0.17	38.83 ± 0.11	41.56 ± 0.30
3	29.06 ± 0.32	37.03 ± 1.00	36.40 ± 0.80	34.93 ± 0.68	37.76 ± 0.55	41.63 ± 0.20
6	28.93 ± 0.05	36.90 ± 0.45	36.26 ± 0.37	34.23 ± 0.25	37.16 ± 0.40	40.90 ± 0.10
12	28.30 ± 1.05	35.90 ± 0.51	35.80 ± 0.30	33.80 ± 0.91	34.70 ± 0.58	38.06 ± 0.15
24	28.86 ± 0.11	34.10 ± 0.17	33.90 ± 0.10	32.93 ± 0.15	33.20 ± 0.21	36.96 ± 0.32
Person B – Arm						
Time (Hour)	Dry Skin (%)	Pure light water-added moisturiser (%)	Commercial moisturiser (%)	1 % ^{18}O enriched water- added moisturiser (%)	10 % ^{18}O enriched water- added moisturiser (%)	50 % ^{18}O enriched water- added moisturiser (%)
0	29.76 ± 0.28	29.66 ± 0.45	29.66 ± 0.20	29.26 ± 0.85	29.83 ± 0.11	30.33 ± 0.20
0.25	29.50 ± 0.45	45.56 ± 0.92	45.30 ± 0.10	46.20 ± 0.91	49.06 ± 0.20	47.96 ± 1.25
1	30.33 ± 0.37	41.90 ± 0.72	41.80 ± 0.10	41.10 ± 0.95	45.23 ± 1.65	45.40 ± 0.10
3	32.80 ± 0.43	43.26 ± 0.60	43.80 ± 0.10	44.86 ± 0.11	46.06 ± 0.15	47.60 ± 0.30
6	31.86 ± 0.47	43.23 ± 0.90	44.23 ± 0.58	42.30 ± 0.52	43.83 ± 1.43	45.20 ± 1.15
12	31.43 ± 0.28	41.50 ± 0.10	41.96 ± 0.37	40.20 ± 0.45	42.33 ± 1.36	43.83 ± 0.45
24	28.86 ± 0.11	34.10 ± 0.17	32.93 ± 0.45	32.93 ± 0.15	36.03 ± 0.25	36.96 ± 0.32
Person C – Arm						
Time (Hour)	Dry Skin (%)	Pure light water-added moisturiser (%)	Commercial moisturiser (%)	1 % ^{18}O enriched water- added moisturiser (%)	10 % ^{18}O enriched water- added moisturiser (%)	50 % ^{18}O enriched water- added moisturiser (%)
0	35.03 ± 0.60	35.43 ± 0.20	35.33 ± 0.40	34.80 ± 0.20	35.10 ± 0.10	32.76 ± 0.30
0.25	34.03 ± 0.35	47.73 ± 0.96	47.16 ± 0.81	47.10 ± 0.72	51.03 ± 1.70	50.76 ± 1.67
1	33.73 ± 0.49	46.23 ± 0.05	45.23 ± 0.15	43.66 ± 0.68	45.83 ± 0.94	46.83 ± 0.60
3	34.60 ± 0.85	46.66 ± 1.46	46.10 ± 0.10	42.53 ± 0.32	46.26 ± 0.73	47.36 ± 0.80
6	35.96 ± 0.32	40.33 ± 0.20	40.40 ± 0.47	41.70 ± 0.88	46.13 ± 0.95	47.83 ± 0.83
12	33.96 ± 1.24	39.46 ± 1.20	38.20 ± 0.20	36.23 ± 0.51	38.83 ± 0.70	39.70 ± 1.00
24	35.40 ± 0.20	37.20 ± 0.34	37.00 ± 0.20	34.80 ± 0.10	35.00 ± 0.10	35.20 ± 0.20
Person D – Arm						
Time (Hour)	Dry Skin (%)	Pure light water-added moisturiser (%)	Commercial moisturiser (%)	1 % ^{18}O enriched water- added moisturiser (%)	10 % ^{18}O enriched water- added moisturiser (%)	50 % ^{18}O enriched water- added moisturiser (%)
0	36.23 ± 0.72	36.43 ± 0.32	36.20 ± 0.10	36.16 ± 0.32	36.30 ± 0.43	36.60 ± 0.10
0.25	34.86 ± 0.56	48.53 ± 0.25	48.10 ± 0.10	47.40 ± 0.45	50.73 ± 1.27	52.56 ± 1.46
1	38.06 ± 0.95	44.36 ± 0.70	44.30 ± 0.65	45.63 ± 0.68	47.86 ± 0.40	47.93 ± 0.37
3	41.00 ± 0.51	45.70 ± 0.17	46.00 ± 0.91	46.96 ± 0.47	48.26 ± 0.40	49.93 ± 0.47
6	37.63 ± 0.28	40.93 ± 0.95	41.20 ± 0.20	43.33 ± 0.85	45.30 ± 0.30	45.16 ± 0.47
12	37.53 ± 0.55	40.43 ± 0.32	40.20 ± 0.26	40.60 ± 0.45	43.10 ± 0.10	42.80 ± 0.60
24	37.16 ± 0.76	37.43 ± 0.05	37.20 ± 0.20	38.83 ± 1.11	41.40 ± 0.40	41.60 ± 0.30

Table 3.1.4.3 The moisture increasing on hand areas.

Person A – Hand				
Time (Hour)	Commercial moisturiser (%)	1 % ^{18}O Water added moisturiser (%)	10 % Water added moisturiser (%)	50 % Water added moisturiser (%)
0.25	11.86 ± 0.75	10.53 ± 0.30	13.13 ± 0.15	15.06 ± 0.25
1	8.68 ± 0.05	7.20 ± 0.73	10.33 ± 0.61	10.60 ± 1.01
3	6.03 ± 0.50	5.53 ± 0.25	8.36 ± 0.80	8.46 ± 0.47
6	6.53 ± 1.32	6.43 ± 0.60	10.10 ± 0.72	9.36 ± 1.32
12	1.22 ± 0.91	2.27 ± 0.17	3.10 ± 2.29	7.67 ± 0.20
24	1.72 ± 0.70	1.64 ± 0.85	4.70 ± 0.73	4.04 ± 1.83
Person B –Hand				
Time (Hour)	Commercial moisturiser (%)	1 % ^{18}O Water added moisturiser (%)	10 % Water added moisturiser (%)	50 % Water added moisturiser (%)
0.25	21.20 ± 2.75	19.90 ± 1.99	28.36 ± 1.12	33.96 ± 0.45
1	13.72 ± 1.22	13.67 ± 0.79	20.40 ± 1.84	27.23 ± 0.80
3	14,63 ± 0.90	14.50 ± 0.80	19.43 ± 0.47	24.70 ± 0.97
6	7.25 ± 0.66	7.87 ± 0.10	14.67 ± 1.51	9.27 ± 0.20
12	3.70 ± 0.30	5.06 ± 1.35	4.93 ± 0.58	4.30 ± 0.56
24	1.00 ± 0.20	1.63 ± 0.92	0.96 ± 0.15	1.63 ± 0.15
Person C –Hand				
Time (Hour)	Commercial moisturiser (%)	1 % ^{18}O Water added moisturiser (%)	10 % Water added moisturiser (%)	50 % Water added moisturiser (%)
0.25	21.09 ± 0.77	26.13 ± 0.90	25.93 ± 0.37	29.50 ± 1.24
1	16.23 ± 0.62	20.43 ± 0.73	18.87 ± 0.70	25.07 ± 0.92
3	15.85 ± 0.32	23.20 ± 0.37	20.24 ± 0.51	26.87 ± 0.28
6	12.13 ± 0.25	18.17 ± 0.72	18.70 ± 0.76	22.03 ± 0.55
12	8.88 ± 0.75	10.34 ± 0.90	9.77 ± 1.24	18.84 ± 0.88
24	2.20 ± 0.30	4.90 ± 0.10	5.20 ± 0.20	6.20 ± 0.20
Person D –Hand				
Time (Hour)	Commercial moisturiser (%)	1 % ^{18}O Water added moisturiser (%)	10 % Water added moisturiser (%)	50 % Water added moisturiser (%)
0.25	14.42 ± 0.20	17.60 ± 1.20	15.67 ± 0.68	20.20 ±1.63
1	11.70 ± 0.30	12.60 ± 0.64	13.97 ± 0.80	17.40 ± 0.57
3	6.33 ± 1.34	8.80 ± 0.86	9.47 ± 1.50	13.97 ± 0.87
6	4.65 ± 0.73	9.43 ± 0.20	11.67 ± 0.78	13.00 ± 0.15
12	0.77 ± 1.00	0.47 ± 0.36	2.27 ± 0.20	1.17 ± 0.10
24	0.77 ± 0.90	6.34 ± 0.20	5.47 ± 0.58	6.74 ± 0.20

Table 3.1.4.4 The moisture increasing on arm areas.

Person A –Arm				
Time (Hour)	Commercial moisturiser (%)	1 % ^{18}O Water added moisturiser (%)	10 % Water added moisturiser (%)	50 % Water added moisturiser (%)
0.25	14.83 ± 0.30	14.17 ± 0.70	15.8 ± 0.17	21.05 ± 0.62
1	13.00 ± 0.30	11.77 ± 0.17	14.10 ± 0.11	16.83 ± 0.30
3	7.65 ± 0.80	5.87 ± 0.68	8.70 ± 0.55	12.57 ± 0.20
6	7.65 ± 0.37	5.30 ± 0.25	8.23 ± 0.40	11.97 ± 0.10
12	7.55 ± 0.30	5.50 ± 0.91	6.40 ± 0.58	9.76 ± 0.15
24	5.14 ± 0.10	4.07 ± 0.15	4.34 ± 0.21	8.10 ± 0.32
Person B –Arm				
Time (Hour)	Commercial moisturiser (%)	1 % ^{18}O Water added moisturiser (%)	10 % Water added moisturiser (%)	50 % Water added moisturiser (%)
0.25	15.93 ± 0.10	16.70 ± 0.91	19.56 ± 0.20	18.38 ± 1.25
1	11.52 ± 0.10	10.77 ± 0.95	14.90 ± 1.65	15.07 ± 0.10
3	10.90 ± 0.10	12.06 ± 0.11	13.26 ± 0.15	14.80 ± 0.30
6	11.87 ± 0.58	10.44 ± 0.52	11.97 ± 1.43	13.34 ± 1.15
12	10.30 ± 0.37	8.77 ± 0.45	10.90 ± 1.36	12.40 ± 0.45
24	4.65 ± 0.45	4.07 ± 0.15	7.17 ± 0.25	8.10 ± 0.32
Person C –Arm				
Time (Hour)	Commercial moisturiser (%)	1 % ^{18}O Water added moisturiser (%)	10 % Water added moisturiser (%)	50 % Water added moisturiser (%)
0.25	13.41 ± 0.81	13.07 ± 0.72	17.00 ±1.70	16.73 ± 1.67
1	12.00 ± 0.15	9.93 ± 0.68	12.10 ± 0.94	13.10 ± 0.60
3	11.78 ± 0.10	7.93 ± 0.32	11.66 ± 0.73	12.76 ± 0.80
6	4.40 ± 0.47	5.74 ± 0.88	10.17 ± 0.95	11.87 ± 0.83
12	4.87 ± 0.20	2.27 ± 0.51	4.87 ± 0.70	5.74 ± 1.00
24	1.70 ± 0.20	-0.60 ± 0.10	-0.40 ± 0.10	-0.20 ± 0.20
Person D –Arm				
Time (Hour)	Commercial moisturiser (%)	1 % ^{18}O Water added moisturiser (%)	10 % Water added moisturiser (%)	50 % Water added moisturiser (%)
0.25	11.43 ± 0.10	12.54 ± 0.45	15.87 ± 1.27	17.70 ± 1.46
1	6.27 ± 0.65	7.57 ± 0.68	9.80 ± 0.40	9.87 ± 0.37
3	4.85 ± 0.91	5.96 ± 0.47	7.26 ± 0.40	8.93 ± 0.47
6	3.43 ± 0.20	5.70 ± 0.85	7.67 ± 0.30	7.53 ± 0.47
12	2.78 ± 0.26	3.07 ± 0.45	5.57 ± 0.10	5.27 ± 0.60
24	0.15 ± 0.20	1.67 ± 1.11	4.24 ± 0.40	4.17 ± 0.30

Table 3.1.4.5 The measurements for different time periods on hand areas.

Person	Commercial moisturiser (%)	1 % ¹⁸O enriched water added-moisturiser (%)	10 % ¹⁸O enriched water added-moisturiser (%)	50 % ¹⁸O enriched water added-moisturiser (%)
Hand – 15ᵗʰ minute				
A	11.86 ± 0.75	10.53 ± 0.30	13.13 ± 0.15	15.06 ± 0.25
B	21.20 ± 2.75	19.90 ± 1.99	28.36 ± 1.12	33.96 ± 0.45
C	21.09 ± 0.77	26.13 ± 0.90	25.93 ± 0.37	29.50 ± 1.24
D	14.42 ± 0.20	17.60 ± 1.20	15.67 ± 0.68	20.20 ±1.63
Average	**17.14**	**18.54**	**20.77**	**24.68**
Hand – 1ˢᵗ hour				
A	8.68 ± 0.05	7.20 ± 0.73	10.33 ± 0.61	10.60 ± 1.01
B	13.72 ± 1.22	13.67 ± 0.79	20.40 ± 1.84	27.23 ± 0.80
C	16.23 ± 0.62	20.43 ± 0.73	18.87 ± 0.70	25.07 ± 0.92
D	11.70 ± 0.30	12.60 ± 0.64	13.97 ± 0.80	17.40 ± 0.57
Average	**12.58**	**13.47**	**15.89**	**20.07**
Hand – 3ʳᵈ hour				
A	6.03 ± 0.50	5.53 ± 0.25	8.36 ± 0.80	8.46 ± 0.47
B	14,63 ± 0.90	14.50 ± 0.80	19.43 ± 0.47	24.70 ± 0.97
C	15.85 ± 0.32	23.20 ± 0.37	20.24 ± 0.51	26.87 ± 0.28
D	6.33 ± 1.34	8.80 ± 0.86	9.47 ± 1.50	13.97 ± 0.87
Average	**10.71**	**13.00**	**14.37**	**18.50**
Hand – 6ᵗʰ hour				
A	6.53 ± 1.32	6.43 ± 0.60	10.10 ± 0.72	9.36 ± 1.32
B	7.25 ± 0.66	7.87 ± 0.10	14.67 ± 1.51	9.27 ± 0.20
C	12.13 ± 0.25	18.17 ± 0.72	18.70 ± 0.76	22.03 ± 0.55
D	4.65 ± 0.73	9.43 ± 0.20	11.67 ± 0.78	13.00 ± 0.15
Average	**7.64**	**10.47**	**13.78**	**13.41**
Hand – 12ᵗʰ hour				
A	1.22 ± 0.91	2.27 ± 0.17	3.10 ± 2.29	7.67 ± 0.20
B	3.70 ± 0.30	5.06 ± 1.35	4.93 ± 0.58	4.30 ± 0.56
C	8.88 ± 0.75	10.34 ± 0.90	9.77 ± 1.24	18.84 ± 0.88
D	0.77 ± 1.00	0.47 ± 0.36	2.27 ± 0.20	1.17 ± 0.10
Average	**3.64**	**4.53**	**5.01**	**7.99**
Hand – 24ᵗʰ hour				
A	1.72 ± 0.70	1.64 ± 0.85	4.70 ± 0.73	4.04 ± 1.83
B	1.00 ± 0.20	1.63 ± 0.92	0.96 ± 0.15	1.63 ± 0.15
C	2.20 ± 0.30	4.90 ± 0.10	5.20 ± 0.20	6.20 ± 0.20
D	0.77 ± 0.90	6.34 ± 0.20	5.47 ± 0.58	6.74 ± 0.20
Average	**1.42**	**3.62**	**4.08**	**4.65**

For the indicated measuring times, the average of the moisture of all moisturisers were calculated for four different people.

Table 3.1.4.6 The measurements for different time periods on arm areas.

| Person	Commercial moisturiser (%)	1 % ^{18}O Water added moisturiser (%)	10 % ^{18}O Water added moisturiser (%)	50 % ^{18}O Water added moisturiser (%)
Arm – 15th minute				
A	14.83 ± 0.30	14.17 ± 0.70	15.8 ± 0.17	21.05 ± 0.62
B	15.93 ± 0.10	16.70 ± 0.91	19.56 ± 0.20	18.38 ± 1.25
C	13.41 ± 0.81	13.07 ± 0.72	17.00 ± 1.70	16.73 ± 1.67
D	11.43 ± 0.10	12.54 ± 0.45	15.87 ± 1.27	17.70 ± 1.46
Average	**13.90**	**14.12**	**17.05**	**18.46**
Arm – 1st hour				
A	13.00 ± 0.30	11.77 ± 0.17	14.10 ± 0.11	16.83 ± 0.30
B	11.52 ± 0.10	10.77 ± 0.95	14.90 ± 1.65	15.07 ± 0.10
C	12.00 ± 0.15	9.93 ± 0.68	12.10 ± 0.94	13.10 ± 0.60
D	6.27 ± 0.65	7.57 ± 0.68	9.80 ± 0.40	9.87 ± 0.37
Average	**10.69**	**10.01**	**12.72**	**13.71**
Arm – 3rd hour				
A	7.65 ± 0.80	5.87 ± 0.68	8.70 ± 0.55	12.57 ± 0.20
B	10.90 ± 0.10	12.06 ± 0.11	13.26 ± 0.15	14.80 ± 0.30
C	11.78 ± 0.10	7.93 ± 0.32	11.66 ± 0.73	12.76 ± 0.80
D	4.85 ± 0.91	5.96 ± 0.47	7.26 ± 0.40	8.93 ± 0.47
Average	**8.79**	**7.95**	**10.22**	**12.26**
Arm – 6th hour				
A	7.65 ± 0.37	5.30 ± 0.25	8.23 ± 0.40	11.97 ± 0.10
B	11.87 ± 0.58	10.44 ± 0.52	11.97 ± 1.43	13.34 ± 1.15
C	4.40 ± 0.47	5.74 ± 0.88	10.17 ± 0.95	11.87 ± 0.83
D	3.43 ± 0.20	5.70 ± 0.85	7.67 ± 0.30	7.53 ± 0.47
Average	**6.83**	**6.79**	**9.51**	**11.17**
Arm – 12th hour				
A	7.55 ± 0.30	5.50 ± 0.91	6.40 ± 0.58	9.76 ± 0.15
B	10.30 ± 0.37	8.77 ± 0.45	10.90 ± 1.36	12.40 ± 0.45
C	4.87 ± 0.20	2.27 ± 0.51	4.87 ± 0.70	5.74 ± 1.00
D	2.78 ± 0.26	3.07 ± 0.45	5.57 ± 0.10	5.27 ± 0.60
Average	**6.37**	**4.90**	**6.93**	**8.29**
Arm – 24th hour				
A	5.14 ± 0.10	4.07 ± 0.15	4.34 ± 0.21	8.10 ± 0.32
B	4.65 ± 0.45	4.07 ± 0.15	7.17 ± 0.25	8.10 ± 0.32
C	1.70 ± 0.20	-0.60 ± 0.10	-0.40 ± 0.10	-0.20 ± 0.20
D	0.15 ± 0.20	1.67 ± 1.11	4.24 ± 0.40	4.17 ± 0.30
Average	**2.91**	**2.30**	**3.83**	**5.04**
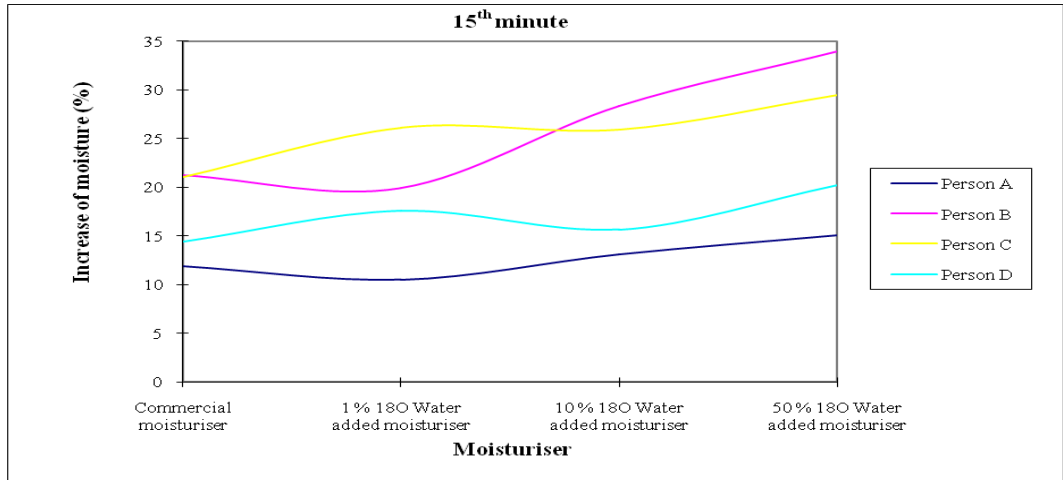
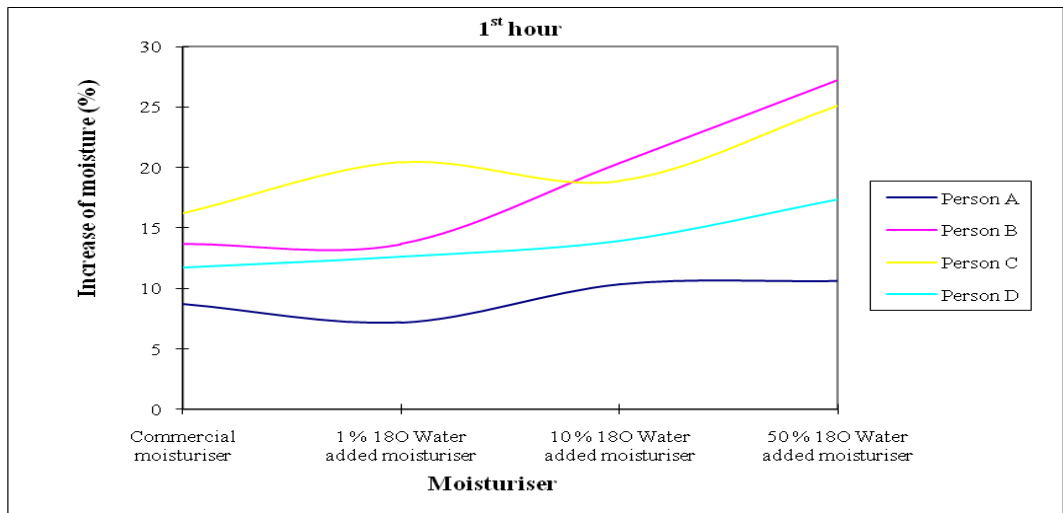**Figure 3.1.4.1** The measurement of hand skin for 15th minute.

Figure 3.1.4.2 The measurement of hand skin for 1st hour.
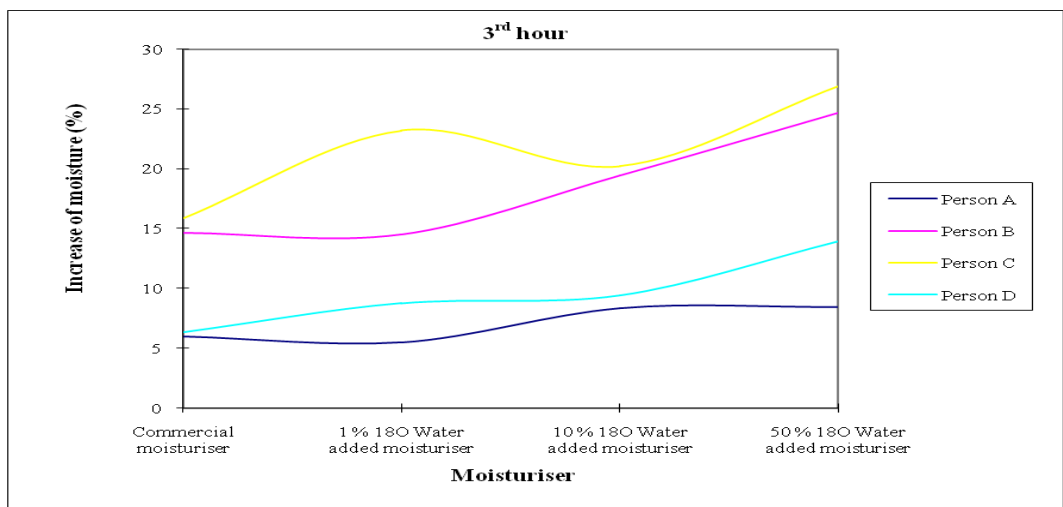

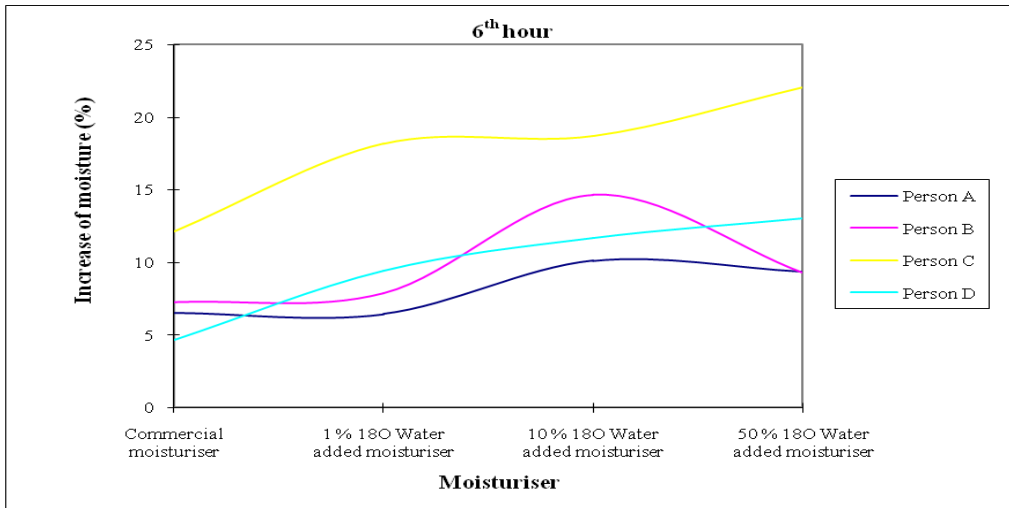**Figure 3.1.4.3** The measurement of hand skin for 3rd hour.

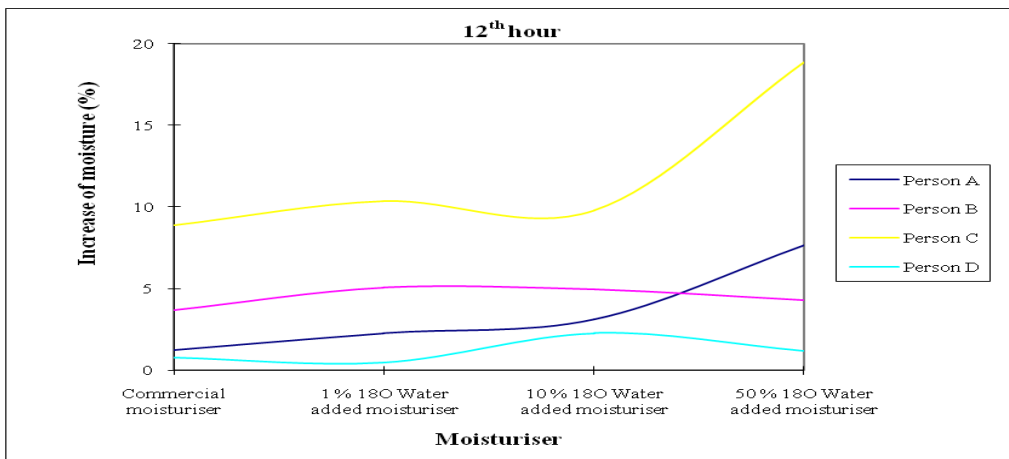**Figure 3.1.4.4** The measurement of hand skin for 6th hour.

Figure 3.1.4.5 The measurement of hand skin for 12th hour.
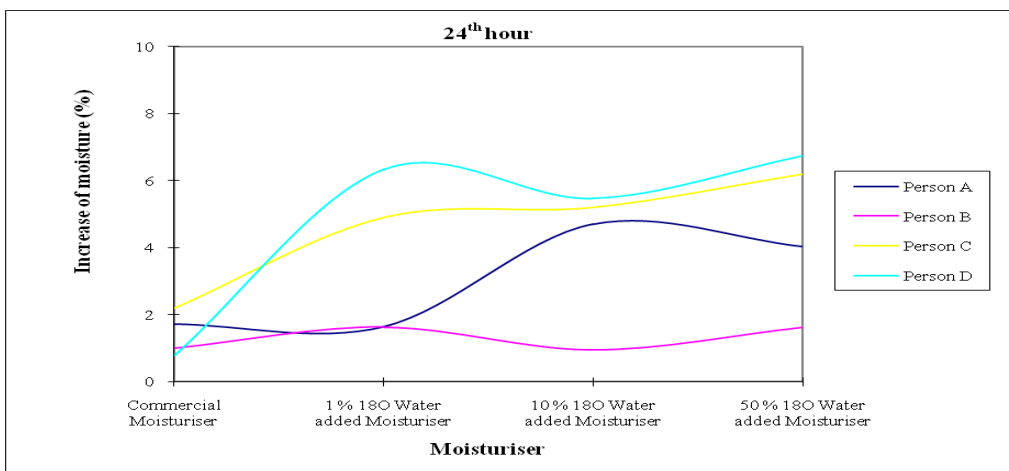

Figure 3.1.4.6 The measurement of hand skin for 24th hour.
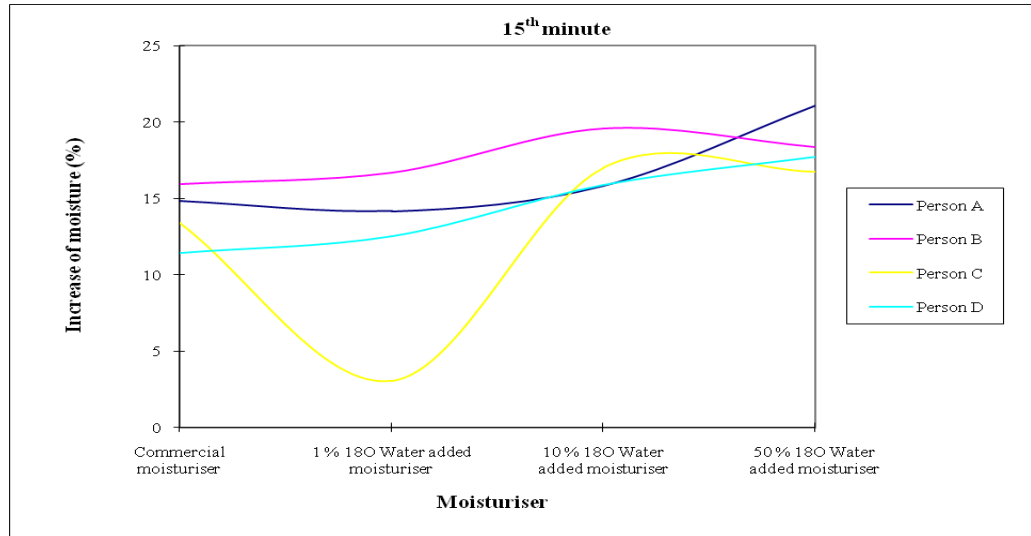
Figure 3.1.4.7 The measurement of arm skin for 15th minute.
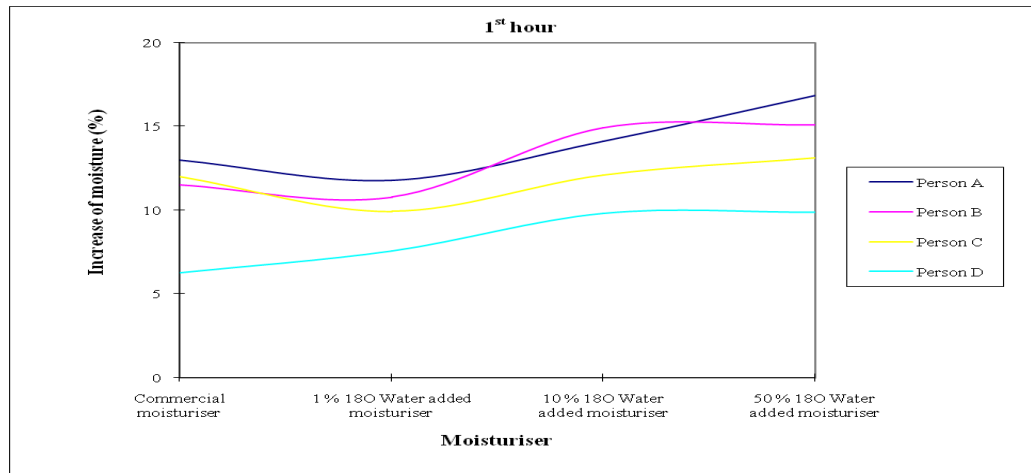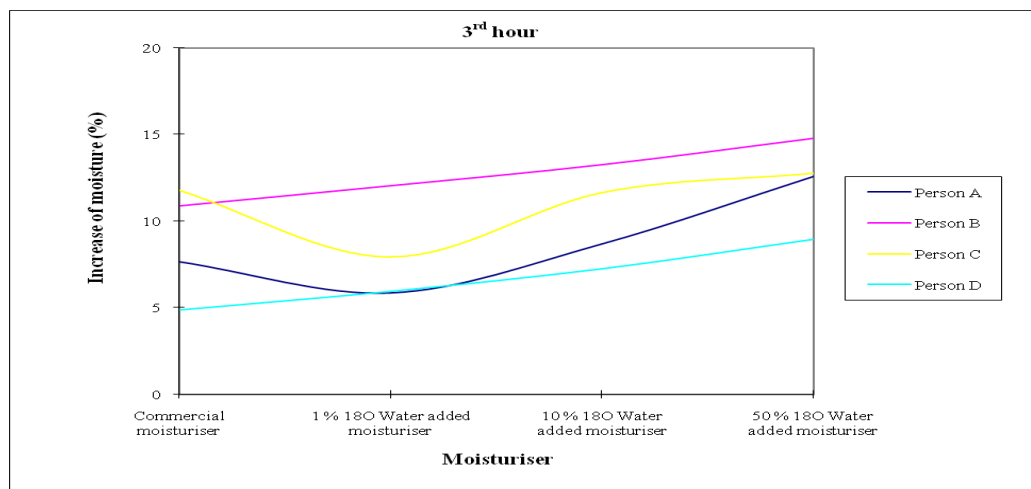

Figure 3.1.4.8 The measurement of arm skin for 1th hour.

Figure 3.1.4.9 The measurement of arm skin for 3th hour.
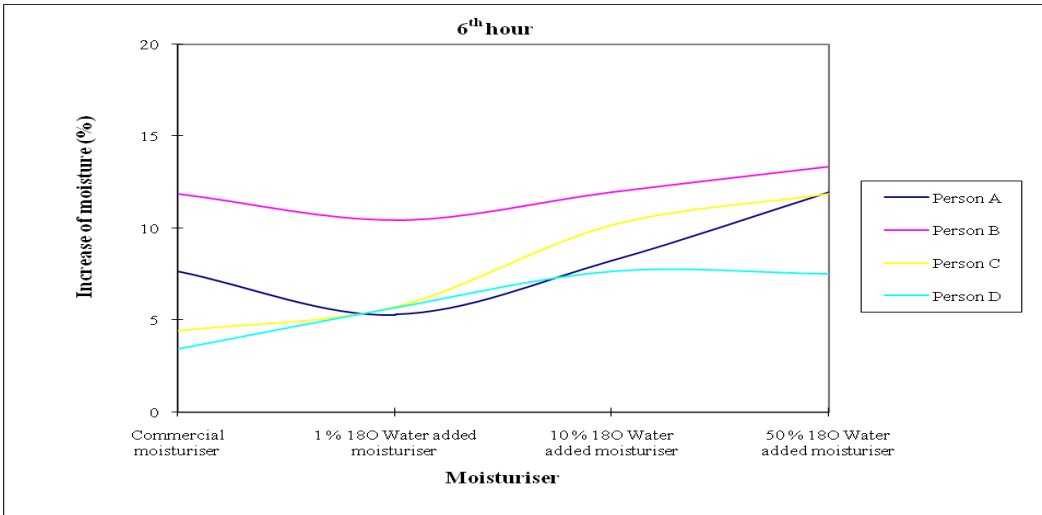
Figure 3.1.4.10 The measurement of arm skin for 6th hour.
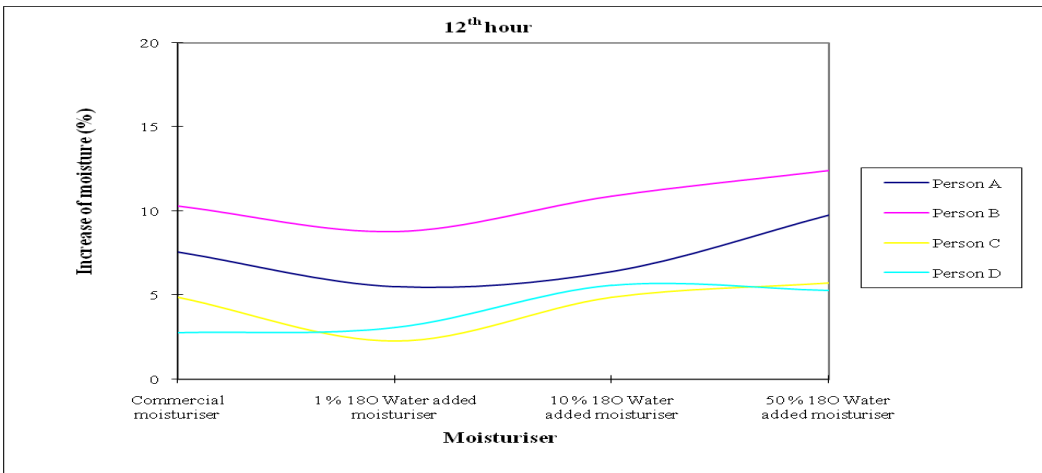

Figure 3.1.4.11 The measurement of arm skin for 12th hour.
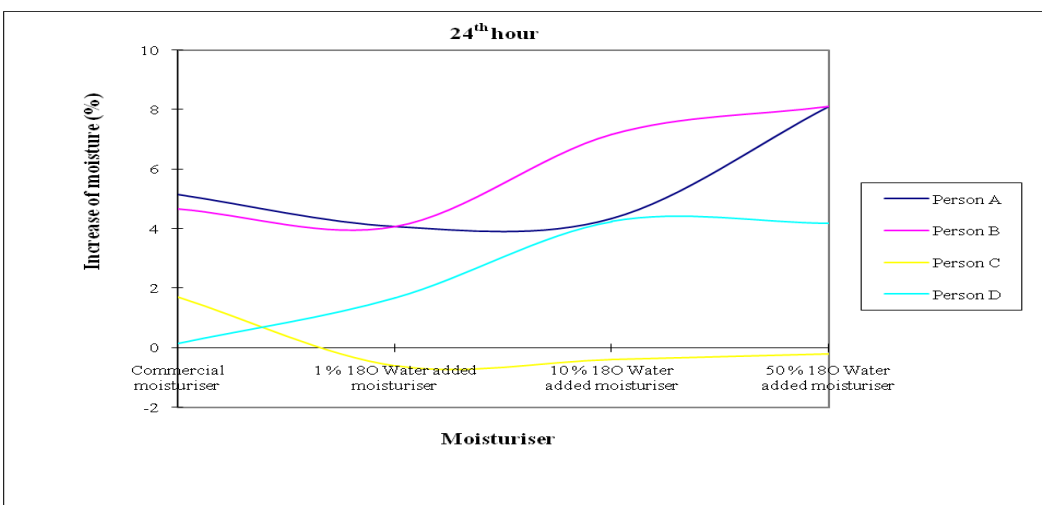

Figure 3.1.4.12 The measurement of arm skin for 24th hour.

Table 3.1.4.7 The average increasing of moisture on hand and arm areas of four different people.

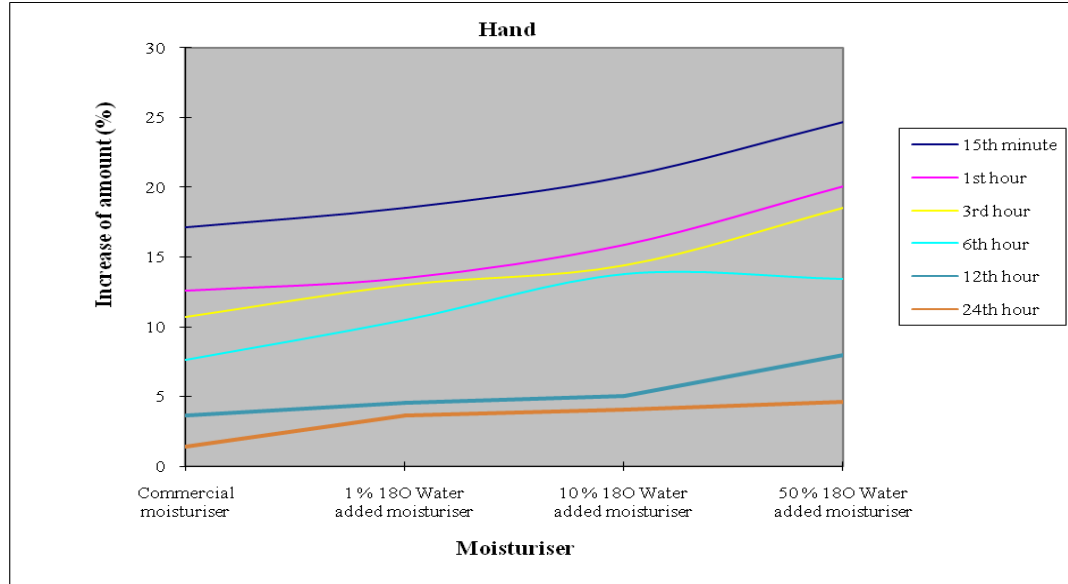| Hand				
Time (Hour)	Commercial moisturiser (%)	1 % ^{18}O enriched water-added moisturiser (%)	10 % ^{18}O enriched water-added moisturiser (%)	50 % ^{18}O enriched water - added moisturiser (%)
0.25	17.14	18.54	20.77	24.68
1	12.58	13.47	15.89	20.07
3	10.71	13.00	14.37	18.50
6	7.64	10.47	13.78	13.41
12	3.64	4.53	5.01	7.99
24	1.42	3.62	4.08	4.65
Arm				
Time (Hour)	Commercial moisturiser (%)	1 % ^{18}O enriched water-added moisturiser (%)	10 % ^{18}O enriched water-added moisturiser (%)	50 % ^{18}O enriched water - added moisturiser (%)
0.25	13.90	14.12	17.05	18.46
1	10.69	10.01	12.72	13.71
3	8.79	7.95	10.22	12.26
6	6.83	6.79	9.51	11.17
12	6.37	4.90	6.93	8.29
24	2.91	2.30	3.83	5.04

Figure 3.1.4.13 The average increasing of moisture on hand skin of four different people.
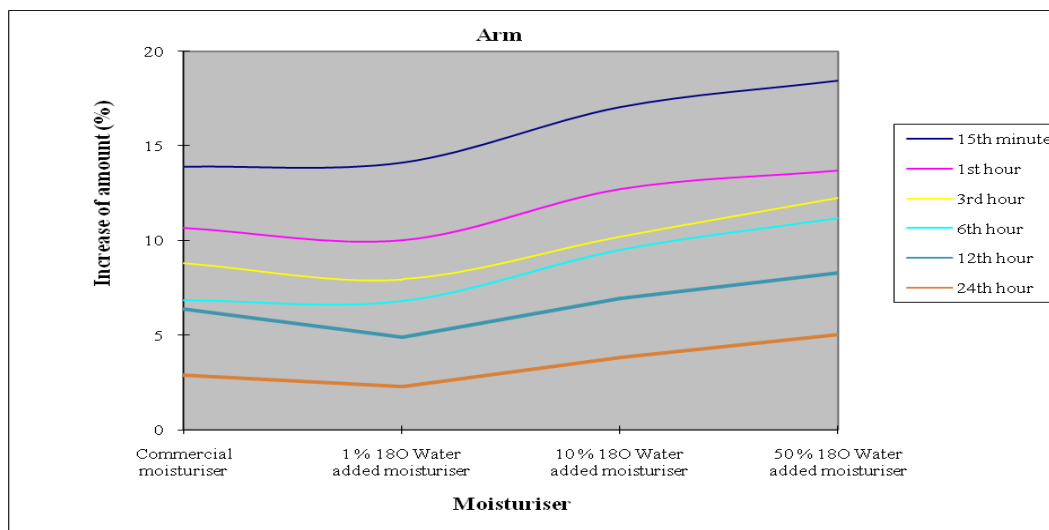
Figure 3.1.4.14 The average increasing of moisture on arm skin of four different people.

Measurements taken from the areas on the hand and arm show that the moisturisers were effective after 15 minutes from the application over the skin and their effect continued for 24 hours.

For the measurement taken over the hand areas, the results showed that the ^{18}O enriched water-added moisturiser increased the moisture percent of the skin more than the commercial moisturiser. The most moisturising effect was seen for the moisturiser prepared with 50 % ^{18}O enriched water. The second most moisturising effect was for the moisturiser prepared with 10 % ^{18}O enriched water. The moisturiser prepared with 1 % ^{18}O enriched water was less effective than these moisturisers, but showed better results than the commercial moisturiser. The measurements taken at 6 and 24 hours showed that 10 % and 50 % enriched moisturiser have similar results and higher values than the other moisturisers.

For the measurements taken over the arm areas, the results showed that the most moisturising effect was the moisturiser prepared with 50 % ^{18}O enriched water. The second moisturising effect was the moisturiser prepared with 10 % ^{18}O enriched water. The results for the commercial and 1 % ^{18}O enriched water-added moisturiser have the similar values. These effects have continued for 24 hours.

3.2 Results of the Cell Culture Studies:

As shown in Table 3.2 and Figure 3.2 over the periods of 24 and 48 hours, the media which was prepared by 1 %, 10 %, and 50 % ^{18}O enriched water did not significantly affect the vitality of the cells and the cells maintained their levels.

Table 3.2 The absorbance values and the vitality percent.

| Time (Hour)		Pure Light Water	1 % $H_2^{18}O$	10 % $H_2^{18}O$	50 % $H_2^{18}O$
24	Absorbance 1	0.749	0.72	0.737	0.68
	Absorbance 2	0.713	0.738	0.759	0.762
	Absorbance 3	0.756	0.753	0.748	0.76
	Avarage Absorbance	0.74	0.74	0.75	0.73
	Blind Absorbance Avarage	0.30	0.29	0.30	0.29
	Standard Deviation	0.023	0.017	0.011	0.047
	Vitality Percent	100	99.21	102.93	98.20
Time (Hour)		Pure Light Water	1 % $H_2^{18}O$	10 % $H_2^{18}O$	50 % $H_2^{18}O$
48	Absorbance 1	0.813	0.85	0.951	0.783
	Absorbance 2	0.988	0.886	0.802	0.748
	Absorbance 3	0.929	0.905	0.786	0.78
	Avarage Absorbance	0.91	0.88	0.85	0.77
	Blind Absorbance Avarage	0.48	0.45	0.42	0.34
	Standard Deviation	0.089	0.028	0.091	0.019
	Vitality Percent	100	93.82	86.75	70.92
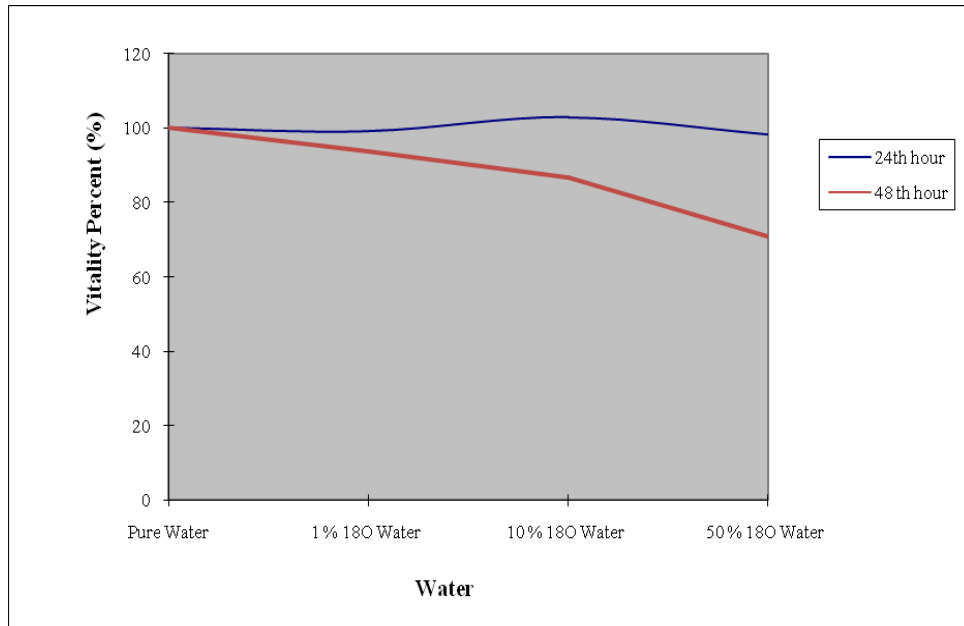
Figure 3.1 The absorbance values and the vitality percent.

4. CONCLUSIONS

Cell culture results obtained in this study have clearly shown that ^{18}O enriched water has not a negative effect on skin cells of mouse. This means that similar results will probably be valid also for human skin cells; but, this should be tested for further investigations on this field. In addition, all other results obtained on moisture measurements on human skin of hand and arm have also clearly shown that moistures which were prepared with ^{18}O enriched water up to about 50 % enrichment level have generally a positive influence on the moisturizing potential of commercial moisturizers. This also indicates that in case of using of ^{18}O enriched water instead of light water for the preparation of commercial moisturizers, the moisturizing quality and health effect of moisturizers will be improved. Surely, more detailed investigations in this field should be carried out.

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