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Studies on molar extinction coefficients of some bio-molecules

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ABSTRACT

Molar extinction coefficients of bio-molecules such as, amino acids (glycine, alanine, serine, cysteine, aspartic acid, asparagine, threonine, proline, glutamic acid, glutamine, valine, methionine, histidine, leucine, lysine, arginine, phenylalanine, tyrosine, tryptophan), fatty acids (lauric, myristic, palmitic, stearic, arachidic, behenic, lignoceric, cerotic, montanic, palmitoleic, oleic, brassidic, nervonic, linoleic, linolenic, arachidonic, eicosapentaenoic, docosahexenoic), and carbohydrates (glyceraldehyde, erythrose, arabinose, glucose, sucrose, raffinose) have been determined in the extended photon-energy region 1 keV to 100 GeV. Calculations have been carried out using a computer program (WinXCom) based on a modern data base of photon interaction cross-sections. It is found that, different bio-molecules having the same molecular formula have same molar extinction coefficient values varying within small uncertainty. These coefficients have been found to depend upon the photon energy following a nine-parameter polynomial. It is also found that these coefficients are independent of the nature of the binding between the various atoms and depend mainly on the number and nature of atoms. Theoretical values for the molar extinction coefficient are compared with experimental values. A good agreement has been obtained between the theoretical values and experimental results.

KEYWORDS

Molar extinction coefficient;
Mass attenuation coefficient;
Interaction cross-section;
Bio-molecules;
Macromolecules;
Amino acids;
Fatty acids;
Carbohydrates.

INTRODUCTION

The study of absorption of gamma radiations in materials of biological importance has been an important subject in the field of radiation physics and is potentially useful in the development of semi-empirical formulations[1]. Since gamma-active isotopes are used in medicine, biological studies, research and agriculture and industry etc.[1,2], a thorough knowledge of the interaction of photons with biologically important substances such as carbohydrates, amino acids and fatty acids is desirable. Amino acids are the main components of biological membrane. Protein is needed by every living organism, and next to water, makes up the largest portion of our body weight since it is present in muscles, organs, hair etc. Amino acids are the building blocks of proteins, which are the most abundant macromolecules in living cells and constitute the largest living matter in all types of cells. One of the most important classes of compounds used by plant and animal systems for energy storage consists of fats and oils. Hydrolysis of a fat or an oil yields glycerol and carboxylic acids, the latter commonly known as fatty acids. Carbohydrates are the most abundant class of biological molecules found in all living organisms. Carbohydrates include sugars, starch, cellulose and many other compounds

TABLE 1: Molecular formulae of bio-molecules studied in the present work

S.no.	Amino acids
1	Glycine ($C_2H_5O_2N$)
2	Alanine ($C_3H_7O_2N$)
3	Serine ($C_3H_7O_3N$)
4	Cysteine ($C_3H_7O_2NS$)
5	Aspartic acid ($C_4H_7O_4N$)
6	Asparagine ($C_4H_8O_3N_2$)
7	Threonine ($C_4H_9O_3N$)
8	Proline ($C_5H_9O_2N$)
9	Glutamic acid ($C_5H_9O_4N$)
10	Glutamine ($C_5H_{10}O_3N_2$)
11	Valine ($C_5H_{11}O_2N$)
12	Methionine ($C_5H_{11}O_2NS$)
13	Histidine ($C_6H_9O_2N_3$)
14	Leucine ($C_6H_{13}O_2N$)
15	Lysine ($C_6H_{14}O_2N_2$)
16	Arginine ($C_6H_{14}O_2N_4$)
17	Phenylalanine($C_9H_{11}O_2N$)
18	Tyrosine ($C_9H_{11}O_3N$)
19	Tryptophan ($C_{11}H_{12}O_2N_2$)
	Fatty acids
20	Lauric acid ($C_{12}H_{24}O_2$)
21	Myristic acid ($C_{14}H_{28}O_2$)
22	Palmitic acid ($C_{16}H_{32}O_2$)
23	Stearic acid ($C_{18}H_{36}O_2$)
24	Arachidic acid ($C_{20}H_{40}O_2$)
25	Behenic acid ($C_{22}H_{44}O_2$)
26	Lignoceric acid ($C_{24}H_{48}O_2$)
27	Cerotic acid ($C_{26}H_{52}O_2$)
28	Montanic acid ($C_{28}H_{56}O_2$)
29	Palmitoleic acid ($C_{16}H_{30}O_2$)
30	Oleic acid ($C_{18}H_{34}O_2$)
31	Brassidic acid ($C_{22}H_{30}O_2$)
32	Nervonic acid ($C_{24}H_{36}O_2$)
33	Linoleic acid ($C_{18}H_{32}O_2$)
34	Linolenic acid ($C_{18}H_{30}O_2$)
35	Arachidonic acid ($C_{20}H_{32}O_2$)
36	Eicosapentaenoic acid ($C_{20}H_{30}O_2$)
37	Docosahexenoic acid($C_{22}H_{26}O_2$)
	Carbohydrates
38	Glyceraldehyde($C_3H_6O_3$)
39	Erythrose ($C_4H_8O_4$)
40	Arabinose ($C_5H_{10}O_5$)
41	Glucose ($C_6H_{12}O_6$)
42	Sucrose ($C_{12}H_{22}O_{11}$)
43	Raffinose ($C_{18}H_{32}O_{16}$)
44	Starch (($C_6H_{10}O_5)_n$)

found in living organisms. These, in the form of sugar and starch, represent a major part of the total caloric intake for humans, plants, animal life and many micro-organisms, and are necessary for the growth of body tissues.

The molar extinction coefficient,ε, is commonly used by spectroscopists when dealing with the attenuation of a beam of light (ultraviolet, visible or infrared) passing through a solution. The molar extinction coefficient is an important intrinsic parameter and reliable values of this parameter are required in many scientific, engineering and chemical disciplines involving photo interactions. In literature, there are only few reports on molar extinction coefficients for amino acids[3], fatty acids[4] and carbohydrates[5]. Moreover, these reports are restricted to gamma energies below 1330 keV, that too only at some specific energies. This has prompted us to carry out the present work. Thus, the present study was undertaken to get rigorous and exhaustive information on the molar extinction coefficients for total gamma ray interaction above 1330 keV, at which no experimental or theoretical attempts have seem to be available.

In the present work, the values of the molar extinction coefficients of bio-molecules, such as amino acids, fatty acids and carbohydrates (TABLE 1) have been derived in the photon-energy region from 1 keV to 100 GeV, by using total mass attenuation coefficients calculated by the WinXCom program[6]. Wherever possible, the calculated values are compared with published experimental data.

THE METHOD OF COMPUTATION AND THEORETICAL BASIS

A narrow beam of mono-energetic gamma rays with incident intensity I_o, penetrating a layer of material with thickness x, and density ρ, emerges with intensity I, given by the exponential attenuation law (Lambert–Beer law):

$$I=I_0e^{-\mu x}, \quad (1)$$

where μ is the linear attenuation coefficient of the material (cm^{-1}).

The change in the radiation intensity, dI, due to interaction in the medium during its passage through material is given by,

$$-dI = \sigma INdx, \quad (2)$$

where N is the number of molecules per unit volume and σ is the total interaction cross-section of the compound, having the dimensions of area (m^2) called the probability of interaction and may be visualized as the area, which has to be hit by the photons in order to cause interaction.

$$\sigma = \frac{M}{N_A}\left(\frac{\mu}{\rho}\right), \quad (3)$$

where M is the molar mass (g/mol), N_A is the Avogadro number. μ/ρ is the total photon mass attenuation coefficient of the bio-molecules and can be calculated by 'mixture rule'[1],

$$\left(\frac{\mu}{\rho}\right) = \sum w_i \left(\frac{\mu}{\rho}\right)_i , \quad (4)$$

where w_i and $(\mu/\rho)_i$ are the weight fraction and the mass attenuation coefficient of the ith constituent element, respectively. For a chemical compound, the weight fraction, w_i, is given by;

$$w_i \frac{n_i A_i}{\sum n_i A_i} , \quad (5)$$

where n_i and A_i are the number of formula units and the atomic weight of the ith element. The computer program WinXCom[6] has generated theoretical values of the mass attenuation coefficient for the bio-molecules. Equation (2) may be written in terms of molar concentration by using $N = N_A c$,

$$-dI = \sigma I N_A c dx , \quad (6)$$

Integration leads to

$$I = I_0 \exp(-\sigma N_A c x) , \quad (7)$$

This expression is essentially identical to the so called 'Lambert–Beer law' which is used to describe radiation attenuation (eq.1). For practical purposes, the following form is preferred:

$$In\left(\frac{I_0}{I}\right) = -\sigma N_A c x \quad (8)$$

In physical chemistry, eq. (8) is commonly written in the following form[7], known as Beer's law:

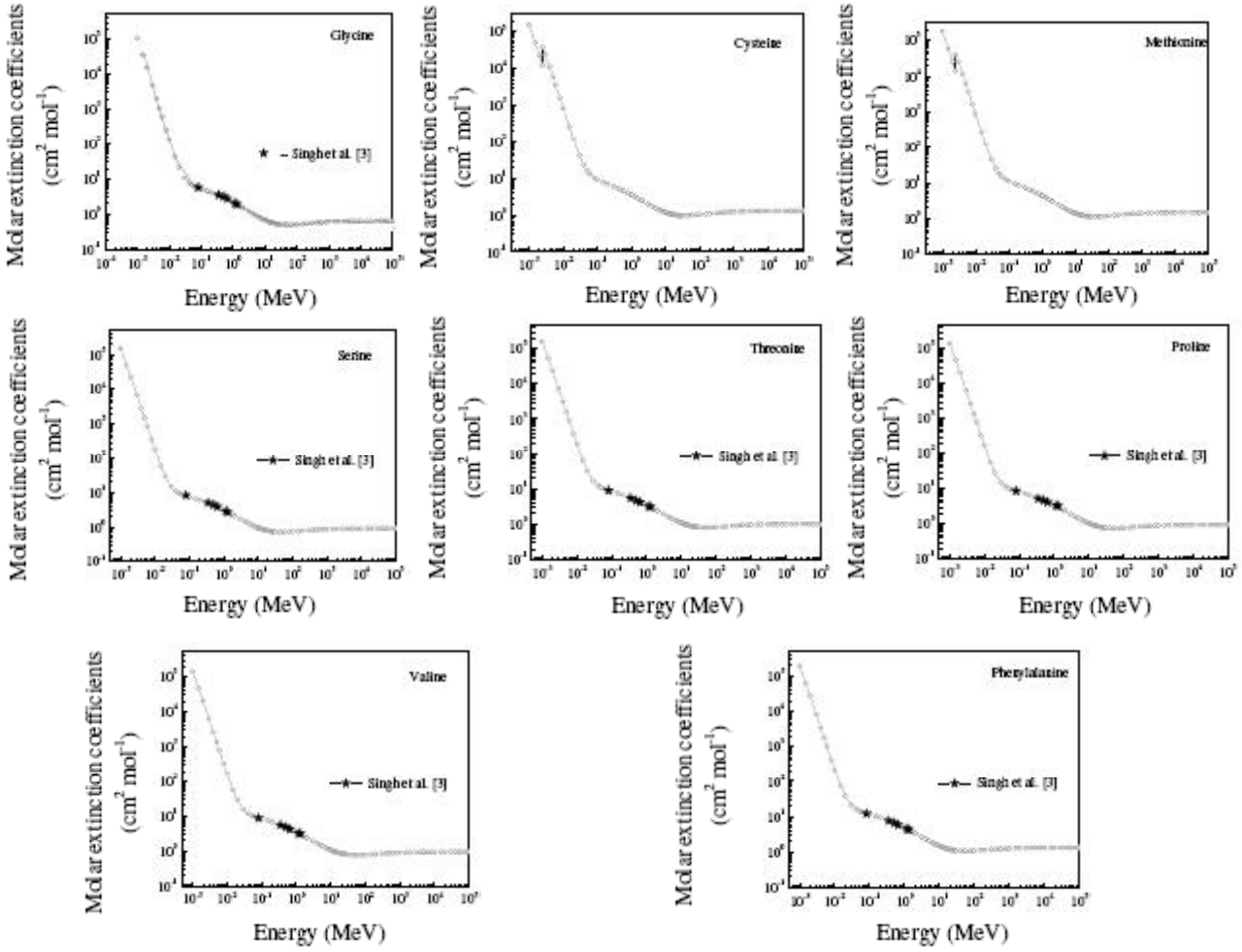

Figure 1: Energy dependence of the molar extinction coefficient of amino acids: (a) Glycine (polynomial was fitted to ε values by a least-squares procedure); (b) Cysteine; (c) Methionine; (d) Serine; (e) Threonine; (f) Proline; (g) Valine; (h) Phenylalanine

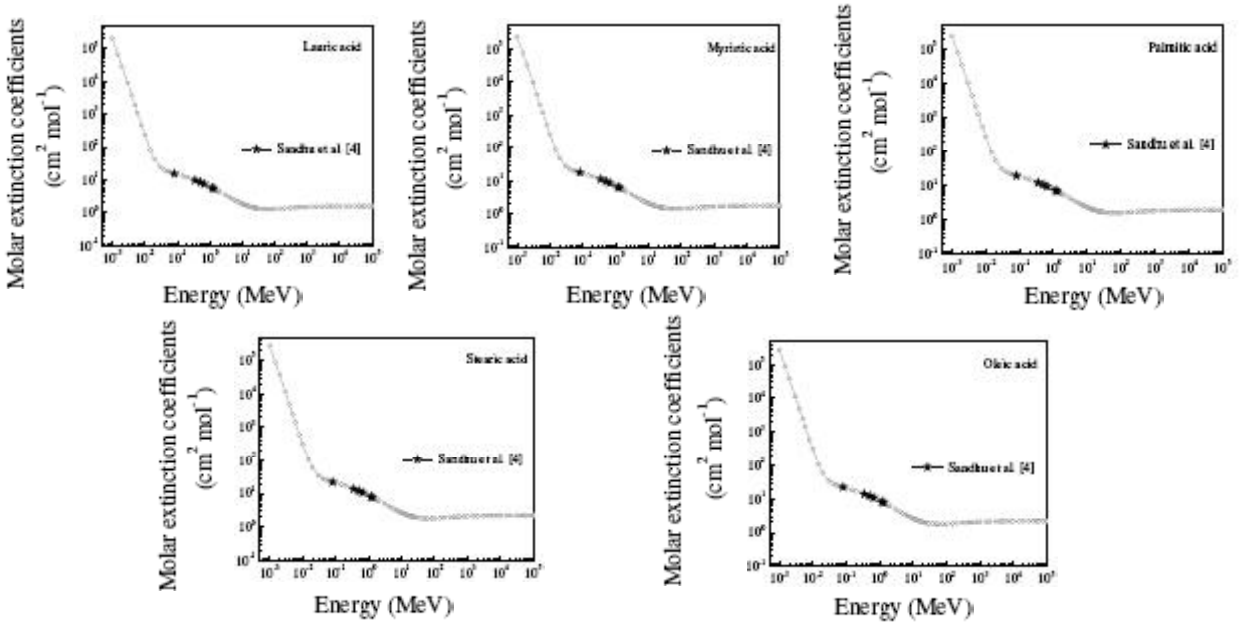

Figure 2 : Energy dependence of the molar extinction coefficient of some fatty acids: (a) Lauric acid; (b) Myristic acid; (c) Palmitic acid; (d) Stearic acid; (e) Oleic acid

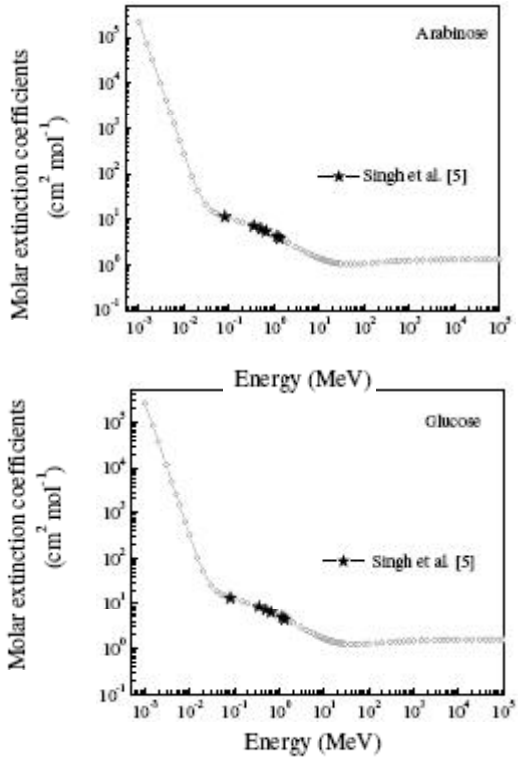

Figure 3: Energy dependence of the molar extinction coefficient of some carbohydrates: (a) Arabinose; (b) Glucose

$$log\left(\frac{I_0}{I}\right) = -\varepsilon cx \qquad (9)$$

where c is the molar concentration of the absorbing species and ε is a constant of proportionality called the molar extinction coefficient. Please note that the base of the logarithm has been changed. Molar extinction coefficient depends upon the wavelength of the incident radiation and is greatest where the absorption is most intense. Its dimensions are 1/(concentration-path length). The molar extinction coefficient is usually expressed in l mol^{-1} cm^{-1}, however the alternative units are cm^2 mol^{-1}. This change in units emphasizes the point that, ε is a molar cross-section for absorption analogous to the mass attenuation coefficient, μ/ρ and the greater the cross-section of the compound for absorption, the greater its ability to block the passage of the incident radiation.

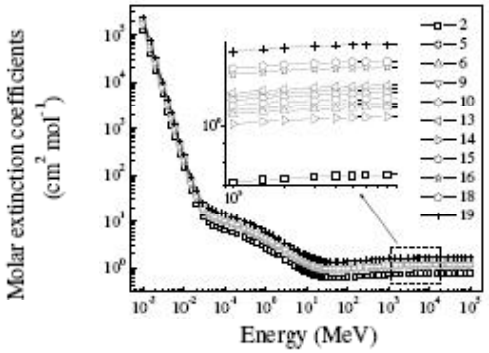

Figure 4: Energy dependence of the molar extinction coefficient of amino acids. The numbers in the graph represent the corresponding molecules (Ref. TABLE 1)

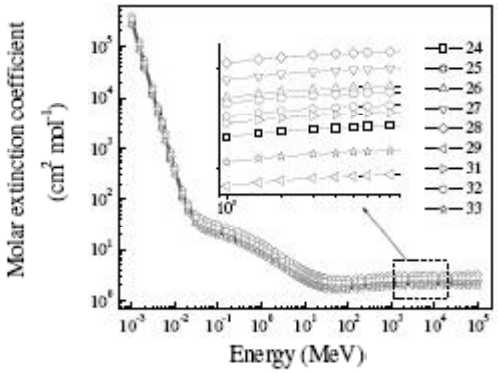

Figure 5: Energy dependence of the molar extinction coefficient of fatty acids. The numbers in the graph represent the corresponding molecules (Ref. TABLE 1)

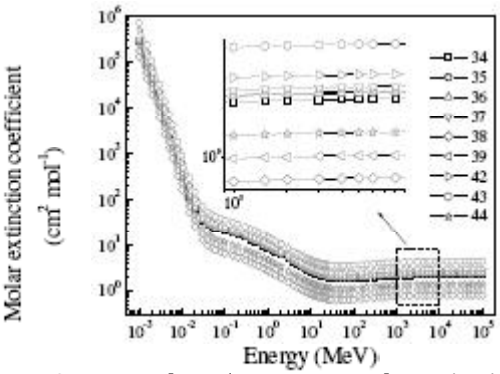

Figure 6: Energy dependence of the molar extinction coefficient of some fatty acids and carbohydrates. The numbers in the graph represent the corresponding molecules (Ref. TABLE 1)

Comparing eqs (8) and (9), we have

$$\varepsilon = \sigma N_A \log_{10} e = 0.4343 \sigma N_A \quad (10)$$

RESULTS AND DISCUSSION

The total mass attenuation coefficients, μ/ρ, of all the biological compounds studied in the present work (TABLE 1) are calculated by using WinXCom computer program. The values of the molar extinction coefficients, ε, are determined by Eq. 10. It is observed from figures 1-6 that, for all the biological compounds, the ε values are very much high at lower energies as compared to the values at high energies. It is because of the fact that, the main contribution to the total interaction cross section is due to incoherent (Compton) scattering. It is due to the presence of low-Z elements such as H, C, N, and O, because for these elements photoelectric and coherent (Rayleigh) scattering cross sections are negligible in comparison to total interaction cross sections. It is also observed that, the variation in ε with chemical composition is large below 10 keV and almost negligible there after.

The variation of the molar extinction coefficient with the photon energy of gamma rays is shown in figure 1a for glycine, stars indicates the experimental values. The energy dependence of molar extinction coefficient mirrors the dominating absorption processes in the various energy ranges. From the figures 1-6 it can be easily seen that, there are three energy regions, where photoelectric absorption, Compton scattering and pair production, respectively, are the dominating attenuation processes. The behavior of all the biological compounds is almost identical except cysteine (Figure 1b) and methionine (Figure 1c). In case of cysteine and methionine, there are two values for molar extinction coefficients at 2.47keV due to the sulfur K-absorption edge. The value 12,396cm^2 mol^{-1} is valid immediately below the absorption edge, and 38,202 cm^2 mol^{-1} immediately above the absorption edge for cysteine. The values 14,081cm^2 mol^{-1} and 39,887cm^2 mol^{-1} are valid immediately below and above the absorption edge for methionine.

The energy dependence of the molar extinction coefficient of all bio-molecules can be described by a 9th degree polynomial:

$$\varepsilon = A + B_1E + B_2E^2 + B_3E^3 + B_4E^4 + B_5E^5 + B_6E^6 + B_7E^7 + B_8E^8 + B_9E^9 \quad (11)$$

where ε is in cm^2 mol^{-1}, E is the energy of photons in MeV, and A and B_i are constants.

For e.g., A typical curve for glycine is shown in figure 1a. In the case of glycine, the constants are $A = 0.33977$, $B_1 = -0.43305$, $B_2 = -0.14861$, $B_3 = 0.03651$, $B_4 = 0.08765$, $B_5 = -0.02697$, $B_6 = -0.00365$, $B_7 = 0.00199$, $B_8 = -1.61153\times10^{-4}$, $B_9 = -2.74426\times10^{-6}$. The results for the other bio-molecules are very similar to the example given here.

Reviewing the data for various amino acids (e.g: leucine and isoleucine: $C_6H_{13}O_2N$) and carbohydrates (e.g: glucose, fructose, galactose, mannose: $C_6H_{12}O_6$; sucrose, maltose, lactose $C_{12}H_{22}O_{11}$; etc.) having the same molecular formula, it is found that the ε values remain the same and are thus independent of the nature of the biological compound. So, in the present approximation, it can be stated that, the molar extinction coefficients are independent of the nature of the binding between the various atoms and depend mainly on the

TABLE 2: Published values of molar extinction coefficients ($cm^2 mol^{-1}$) of some bio-molucules

Bio-molecules		Photon energy (keV)					
		81	356	511	662	1173	1332
Glycine	a	5.80	3.60	3.20	2.80	2.10	1.90
	d	5.66	3.48	3.00	2.68	2.05	1.92
Serine	a	8.40	5.10	4.50	3.90	2.90	2.70
	d	7.93	4.87	4.20	3.76	2.87	2.68
Threonine	a	9.00	5.50	4.70	4.20	3.20	3.10
	d	9.04	5.56	4.81	4.29	3.28	3.07
Proline	a	8.70	5.30	4.70	4.20	3.40	3.30
	d	8.71	5.39	4.65	4.16	3.17	2.97
Valine	a	8.90	5.60	4.90	4.30	3.30	3.10
	d	8.98	5.56	4.80	4.29	3.28	3.07
Phenylalanine	a	12.20	7.70	6.70	5.90	4.60	4.30
	d	12.33	7.65	6.61	5.90	4.50	4.22
Lauric	b	15.60	9.74	8.41	7.51	5.72	5.37
	d	15.60	9.73	8.41	7.51	5.73	5.37
Myristic	b	17.88	11.13	9.63	8.61	6.53	6.14
	d	17.82	11.12	9.61	8.59	6.55	6.13
Palmitic	b	20.06	12.53	10.80	9.64	7.39	6.85
	d	20.03	12.51	10.81	9.66	7.37	6.90
Stearic	b	22.09	13.71	11.87	10.62	8.06	7.56
	d	21.98	13.72	11.86	10.60	8.08	7.57
Oleic	b	22.25	13.92	12.03	10.76	8.18	7.67
	d	22.25	13.90	12.01	10.73	8.19	7.67
Arabinose	c	11.40	7.10	6.10	5.40	4.20	3.80
	d	11.35	6.98	6.01	5.37	4.09	3.83
Glucose	c	13.50	8.50	7.20	6.30	4.90	4.40
	d	13.61	8.34	7.21	6.44	4.91	4.60

a **Experimentally measured values for amino acids (Singh et al., [3]),**
b **Experimentally measured values for fatty acids (Sandhu et al., [4]).**
c **Experimentally measured values for carbohydrates (Singh et al., [5]).**
d **Theoretical values of present investigation obtained using WinXCom.**

number and nature of atoms. This may be due to the fact that, the effect of chemical bonding between the elements is ignored due to the use of additively law[1]. This chemical bonding effect is negligible for photons in the lower energy region. In general, the chemical effect is more significant at higher energies for compounds. Therefore, we feel that the impact of the chemical binding on the molar extinction coefficients of bio-molecules has to be rigorously studied experimentally.

The present theoretical results are in very good agreement with the experimental results of Singh et al.[3] for amino acids, such as glycine, serine, theronine, proline, valine, and phenylalanine. However, the energy range studied by these authors is limited to 81–1332 keV. Sandhu et al.[4] have experimentally determined molar extinction coefficients for some fatty acids in the medium energy range, where Compton scattering is the dominating attenuation process. Singh et al.[5] have made similar studies on some carbohydrates in the energy range 81–1332 keV. Their values are in good agreement with the present calculated values as seen from figures and TABLE 2. The effective atomic numbers in some important amino acids, fatty acids and carbohydrates have been studied by the authors elsewhere[8-10].

CONCLUSION

The molar extinction coefficients of biological compounds, such as amino acids, fatty acids and carbohydrates have been calculated as function of photon energy in the range 1 keV to 100 GeV using WinXCom. The molar extinction coefficients are independent of the nature of the binding between the various atoms and depend mainly on the number and nature of atoms. In literature it has been reported that[3,5], for a particular solute, its molar extinction coefficient remains constant with concentration of the solution and depends upon the wavelength of the incident radiation, and also the molar extinction coefficients of solute in its pure solid form and solutions (solute dissolved in solvent in different proportions) are same. So, the authors suggest that the present results on molar extinction coefficients may be used for applications in scientific, engineering and chemical disciplines.

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