Aqueous Phase Complexation of Heavy Lead (II) Metal Ion With Selected Biomolecules

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Abstract

Considering the underlying principles and theories of coordination chemistry, the chemical basis of the metal-biomoleculeinteractions, the effect of metals and non-metals in their coordinated form, the effect of varying amounts of metals in biochemical pathways,we tried to explore how Lead ion influence its environment and how the environment influence its own properties, particularly accepting the fact thatLead poisoning is probably the most important chronic environmental hazard. Effortswere put to investigate and report the interaction of deleterious Pb (II) ion with selected bioligands, in aqueous phase, which in turn depends on the affinity of Pb(II) with these Bioligands or vice versa, by employing Electrometric techniques. Our main focus has been to calculate stability constants (both the stepwise as well as overall formation constants),the strength of bonding and examine the formation curves by applying Bjerrum's pH – metric titration method as modified by Albert.

Keywords: Bioligands, Biomolecules,Complexes, Coordination Chemistry,Lead (II), Poisoning, Formation Constant.

1. Introduction

Metals play a pivotal role in maintaining the life of all vertebrates. As active centres of metalloproteins, their function is fundamental to our very existence. We cannot think of multistep biological processes (respiration or photosynthesis) without these metalloproteins.The essential role of metal ions in a wide range of biological processes iswell known viz;their selectivelydistribution in biologicalsystems.Considering thethermodynamic and kinetic parameters of the complexes, important properties of metal ions and their toxicities depend on which of thecountless number of possible ligands ends up attached to the metal ion which in turn govern the mode ofbiological action. Byminute changes in the quantities of trace metals,they become potentially toxic which were otherwise essential before an optimal concentration. Lead is an insidiously deceptive poison. Major challenges to the healthprofessionals is its high toxicity potential and the deleterious effectof its early exposure on human health causing oxidative, genetic, metabolic and enzymatic damages. Lead perturbs multiple enzyme systems. As in most heavy metals, any ligand with sulfhydryl groups is vulnerable. Perhaps the best-known effect is that on the production of heme. Due to its charge and ionic radius, Pb (II) is known to replace the essential Cd (II) and Zn (II) in biological system [1-4]. Pb (II) can mimic Ca (II) and access the central nervous system (CNS) through the calcium dependent channels. While in the CNS, it can replace essential calcium cations in protein kinase C isozymes. Calcium (II) can also be replaced by Pb (II) in bones, forming Pb_3 $(PO_4)_2$. Having similar physicochemical properties, Pb^{2+} competes with Ca^{2+} inhibiting the release of neurotransmitters, interfering with the regulation of cell metabolism by binding to calcium receptors and blocking calcium transport to protein binding sites and mitochondria[5]. Lead compounds can induce geno toxicity, neurotoxicity and developmental toxicity in humans. The greatest health concern is the exposure of infants and young children, as well as fatal exposure to lead.The most commonly known effect of lead intoxication is the development of sideroblastic anaemia (loss of normal red blood cell function which causes decreased oxygen delivery to tissues). This anaemia is a result of the decrease in heme synthesis by the inhibition of a Zn (II) dependent enzymed-aminolaevulinic acid dehydratase. Lead is also known to compete with zinc for the zinc binding site of zinc fingers, altering the shape of the protein and destroying its ability to bind DNA[1-3]. Lead is a nonessential element, a ubiquitous environmental contaminant of considerable general and cumulative toxicity, and a suspect human carcinogen. The highly toxic effect of

lead as an environmental pollutant has been known for some years[6] and correlations between lead poisoning[7, 8]and its carcinogenic action in animals have been established[9]. Lead can damage practically all organs, particularly the kidneys and the immune system. The most deceptive and dangerous form of lead poisoning is that affecting the nervous system. In adults, lead damage mainly causes peripheral neuropathy, which is characterized predominantly by demyelination of the nerve fibres. Intense exposure to high lead levels Pb (ll) can cause encephalopathy, with symptoms of vertigo, insomnia, migraine, irritability and even convulsions, seizures and coma. Lower levels of the metal give rise to lead-induced neuropathy, which mainly affects the developing brain and provokes behavioural problems and cognitive impairment. Epidemiological studies have shown a strong correlation between lead levels in blood and bones and poor performance in attitude tests (IQ or psychometric tests). The learning process is based on the creation and remodelling of synapses and the toxic effect of lead on this process suggests that this metal specifically damages the synaptic function. It is, therefore, pertinent toascertain how these metal ions are distributed and concentrated in our bodies and inwhat forms and concentrations they may be harmful and life threatening. Howeverby complexing with appropriate bioligands (like proteins) these toxic metals can beexcreted out of the body which in turn depend on the stability of these metal proteincomplexes. The therapeutic chelators used for the heavy metal toxicity, are not specific for toxic metals alone, they bind both essential as well as toxic metals ions, redistribute toxic effects throughout the body[10] and are themselves potentially toxic. The work described here deals with the interaction of some potential bioligands and their derivatives with Pb (II). The bioligands selected for our investigation are such that in addition to the potential N and O donor sites, some of them have S-atom as an additional donor site. As the chemistry of the metal ions in aqueous phase is actually the chemistry of their aqueous complexes, we thought it proper to investigate their possible role and coordination behaviour in aqueous phase, pH-metrically.Stability constants of the resulting complexes have been determined using Bjerrum's method in aqueous phase [11].

2. Experimental

2.1 Materials and Methods

The Lead saltPb (CH**3**COO**)** **2** used in our study was fromE.Merck, Germany (AR grade). The bioligands, (AR) grade, employed in these investigations were purchased from E. Merck, India(Imidazole , Benzimidazole , Methyl Imidazole Barbituric acid, Thiobarbituric acid, Folic acid, Glutathione, Dopamine) and used as such. The titrant employed was carbonate free KOH (0.1M) solution (Aldrich). After estimating the metals in theirsolutions, the required concentration was maintained by dilution of the stocksolution. All the pH-metric studies were carried out at 20°C. The volume of the alkali (0.1M KOH) used for each titration was 10 ml. Before performing the pH-metric titrations, standard oxalic acid(M/10) solution (E. Merck, India) was used to check the strength of the KOHsolution, periodically.The pH-metric titrations were carried out with digital pH-meter (LABINDIA µP CONTROLLED pH ANALYSER) inconjunction with Orion Gel-Filled combined pH electrode (model 91-06) which was first standardized andcalibrated with pH 4,7 and 9.2 before performing each titration. Conductance measurements were carried out with a conductivity meter (EUTECH INSTRUMENT CON 510). The cell was calibrated with desired KCl solution at 25^0C.

3. Results and Discussion

Depending on the strength of bond between metal ion and the ligand in thermodynamic sense while studying the formation of complexes insolution, the metal-bioligand complexes are generally considered as being stable orunstable.Stability of complex is very often expressed in terms of stability constant, which is used to describe the equilibrium behaviour of metal complexes. In view of the above facts, we have investigated the coordinating behaviourof some biomolecules of biological concern with toxicPb (II) metal ion. Main concern was the calculationof stability constant thereby focussing on the resulting formation curves applying Bjerrum's pH – metric titration method as modified by Albert [11, 12]. It is evident from both the computed stability constants values of the resulting complexes "Table I" and fromboth the pH-

metric titration curves as well as the formation curves “Fig 1-8” of the investigated system that the complexation process has taken place.

Potentiometric /pH-metric Method**:**

The simplest of the electrochemical methods isthat in which the free metal ion concentration is determined in an equilibriumof the type:

$$M^{m+} + nLH \leftrightarrow ML_n^{(m-n)+} + nH^+$$

Since bioligands are either weak acids or weak bases, there is competition between hydrogen ions and metal ions for grabbing these bioligands, which can be used as a basis for the determination of formation constant. Hence the release of hydrogen ions in this coordination reaction can be correlated with the concentration of uncomplexed bioligands and thus, the pH measurement, serves as a suitable method for studying the complex formation phenomena.

Appreciable shifts in pH plots indicated stepwise association of ligands with metal ions. Metal - ligand system may be explained by considering the stepwise formation of complex species of different composition in aqueous solution (ML, ML_2, ML_3--------ML_n, where L stands for ligand and M stands for metal ion and n for number of ligands molecules bound by each metal ion).

In general, at equilibrium the concentration of each species is related to that of each of the other complex species by a series of stepwise formation constant expressions such as;

$$k_1 = [ML]/ [M][L]$$

$$k_2 = [ML_2]/ [ML][L]$$

$$k_3 = [ML_3]/ [ML_2][L]$$

$$k_n = [ML_n]/ [ML_{n-1}][L]$$

K_s, the overall stability constant is related to the stepwise formation constants k_1, k_2,------k_n as: $K_s = k_1k_2k_3$----------k_n

For a divalent metal ion

$K_s = [ML_2]/ [M^{2+}][L]^2$

and for a monovalent metal ion

$K_s = [ML]/ [M^{+}] [L]$

Also $\log K_s = \log k_1 + \log k_2$

When the complex formation starts, H^+ ions are released and the measurement of the concentration of these ions provides a way to determine the extent of complexation of metal ions with a ligand in aqueous phase. The values of stepwise equilibrium constants are given by the following expression

$\log k_1 = \log \bar{n} - \log (1-\bar{n}) - \log [Sc]$

$\log k_2 = \log (\bar{n}-1) - \log (2-\bar{n}) - \log [Sc]$

Here $\bar{n}$ (n bar) is the average number of molecules of complex forming agent attached to one ion of the metal concerned and [Sc] is the concentration of the coordinating species, values of which may be calculated from the equation given below:

$\log [Sc] = (pH-pKa) + \log \{[HSc]^0 - [KOH]\}$

Where $[HSc]^0$ is the concentration of the bioligand before addition of the metal ion and [KOH] is that concentration of alkali (KOH) which would be present if the complex forming agent and the metal ions were both absent i.e.; the initial concentration of KOH. The relationship between n and [KOH] is given by the expression:

$\bar{n} = 2[KOH]/ [HSc]^0$

The values of all these parameters have been computed.

For calculation of $\log k_1$ and $\log k_2$, the most reliable values of [Sc] are found from $\bar{n}$= 0.10 to 0.70 and from 1.30 to 1.70 respectively as when $\bar{n}$ lies between 0.70 and 1.30, some molecules of $(ML)^+$ start to take on another molecules of (L) before all the ligand molecules have interacted with the metallic ions in 1:1 ratio.

Further where $\bar{n}$ is only a small fraction of (1- $\bar{n}$), knife-edge conditions prevail. Finally when n is approaching 2, the conditions are often such that some of the molecules of the complex ML_2 form a weak association with an extra molecule of the ligand. With the help of following relation which is valid only when $\bar{n}$=1, the most reliable value of K_s are obtained from the formation curves.

$K_s=1/ [Sc]^2$

Or $\log K_s= -2\log [Sc]$

By titrating each ligand in the presence of Lead ion, pH-values were obtained for each addition of alkali. With the help of formation curves, the correct values of [Sc] corresponding to $\bar{n}$ = 1 are also found. Having known the value of [Sc], the standard free energy change of complex formation ΔG^o, may also be calculated with the help of the relation:

$\Delta G^0 = - RT \ln K_s$ or $\Delta G^0 = -2.303 RT \log K_s$

Where, R =0.008314 and T=297Kelvin

Since $\Delta G^0 = \Delta H - T\Delta S^0$, $2.303 R \log K_s = (\Delta S^0 - \Delta H^0/T)$

Analysing each metal-bioligand system separately, it is observed that stabilities of the metal complexes cannot be accounted by a single factor rather considered separately in terms of subtle blend ofcontributing factors. Also in case of the mercury lead,their properties may be regarded as a manifestation of the consequences of inert paireffect. As is evident from “Table I”, the stability constants of metal –bioligand systems show a large variation in values. As a general rule higher the value of equilibrium constants, greater is the stability of the resulting metal complexes. In principle, stability constants are determined by studying the concentrations of the various species present in a wide range of equilibrium mixture containing the metal ion and the bioligands in different proportions. An important parameter for

establishing the possibility and extent of formation of a metal-bioligand complex is the disappearance of usually normal chemical properties of the metal ion upon complexation thereby indicating that the free metal ion is present in an extremely low concentration and most of it has complexed with the bioligands. Greater the pKa value of the bioligand, greater will be the stability of the resulting complex indicating that there is a direct dependence of the stability constants of metal-bioligand complexes upon the pKa values of bioligands as is evident from "Table 1". Considering each Lead-Bioligand system, it is seen that the difference in the affinity of various bioligands for Pb (II) ion may be due to several factors such as replacement or substitution of a group, nature of donor atoms, orientation of $-NH_2$, -COOH and R groups, length of carbon chain, involvement of additional coordinating sites/centres, aromatic substitution, acetylation.All these factors play an important role in deciding the extent and mode of complexation of bioligands with Pb (II) ion. In some cases effect of one factor may be counterbalanced or diminished by the effect of some other factors. In fact whole complexation process is pH dependent, which increases with increasing the pH of the solution but decrease near pH 8.3, with the optimal pH region being nearer to physiological pH value. On the basis of HSAB concept of soft-soft interaction, it can be shown that the high thermodynamic stability ofPb (II) with Sulphur containing Bioligands confirms the participation of highly polarisable sulphur atom in the complex formation. The oxygen of an amide group is less electronegative than oxygen of a carbonyl group resulting in the less involvement of amide nitrogen in the bonding as its basicity decreases due to the resonance with carbonyl group. In case of imidazole, benzimidazole and methylimidazole, the presence of non-bonded electron pair on donor nitrogen center that are not involved in resonance isresponsible for their coordination with Pb (II). The conjugate acid of imidazole has apKa of 6.8, it exists in both the protonated and unprotonated forms at physiologicalpH (nearly 7.2). This is one of the reasons for imidazole containing bioligands to bean important component of many enzymes. In case ofbenzimidazole, the resonance effect is responsible for its low stability in comparison to that of imidazole. On introducing methyl group into the imidazole ring, its coordinating ability with Lead ionincreases due to the increase in basicity, as a result of inductive effect. In general as the distance between $-NH_2$ and -COOH groupsincreases, thevalue of log $K_s$decreases. The presence of various donor atoms in Folic acid makes itcapable of acting as a bidentate, tridentate or else as a polydentate ligand. However, due to its bulkier nature and low solubility (in aqueous solution), the resulting Pb (II) complex is not as stable as expected as shown by the stability data. Also folic acid is

similar to dipeptides and like metal-glycylglycine complexes, its stability is lower than that of the simple aminoacid complexes.

Pyrimidines act as bases thereby forming very stable metal coordination complexes. Hence, the bioligands containing pyrimidine bases in their structure can provide a means for coordinating with metals (especially the toxic ones), thereby resulting in their detoxification. In this regard, barbituric acid (2,4,6-trihydroxy pyrimidine) and itsderivative thiobarbituric acid(4,6-dihydroxy-2-mercaptopyrimidine) can serve the purpose. The stability data shows that these bases, like the pyrimidine bases, form very stable Pb (II) metal chelates. However, the stability of thiobarbituric acid with Pb (II) is much greater than the stability of correspondingPb (II) barbituric acid complex in view of the fact that basicity for a particular donor set of chelating agents decreases in the order:

SNN ~ SNO > SO > OO > NO ~ NN

The coordination potential of the bioligands used in our experiments decreased in the same order among all chelating agents. According to the Pearson's HSAB thumb rule, this trend agrees well with the theoretical consideration that sulphur atom being a softbase has more avidity for soft acids, the heavy metals in our case.Thusthiol containing chelating agents (thiobarbituric acid, glutathione) used in our experiments have high stability with the heavy Pb (II) ions.Glutathione, composed of three amino acid residues having four protons that can dissociate as the titration proceeds from acidic to basic region, from pH < 2 to pH=10 regions and at a pH 3 zwitterions species of glutathione exists, plays an important role in cell antioxidant defence mechanism. The stability data reveals that glutathionine have high affinity with Pb (II)ion forming a stable complex. Because of its soft-soft interaction and the tendency to act as bidentate ligand, coordinating through thiol and amide or thiol and carboxylic group, glutathione can be used as an antidote in Pb (II) toxicity. Thus the Molecular weights of chelating agents is another factor affecting the stability of metal complexes. The aliphatic thiol-containing chelating agents like cysteine of low molecular weight, form more stable Pb (II)-complexes than glutathione. On the basis of stability constant data, it can be shown that bioligands containing –SH and -NH_2, or –SH and –COOH group of low molecular weight form more stable complexes. Hence Pb (II) metal ion is of toxicological concerns, especially as environment pollutants, as it is able to inactivate thiol containing enzymes even in low concentration, and thus interfere with cellular metabolisms and functions. The bioligand dopamine has three coordination sites (0, 0, N) two catecholtype (0, 0) and one amine 'N. ThepH plots and the formation curves show that the complexation

process terminates inthe pH range 7.8-8.9 and thus the catechol bonding contributes nothing to thestability of the complex. However the stability data throws light on thebidentate mode (N, 0) of the ligand with the Pb (II) ion.Thus in case of toxicity due to heavy metal ions, it can be proposed that these bioligands can definitelyserve as antidotes.

4 Conclusions

With the help of pH-metric titrations, the interaction of Lead ion with bioligands was studied. In comparison to individual titration curves almost in all the cases, the presence of Pb(II) ion and the bioligand together in the solution causes shift in the titration curves indicating that complexation has taken place. The Formation plots clearly show that the values of n increase with the increase in the pH value, showing thereby, participation of the anionic from of bioligand in the complex formation. Due to the formation of hydroxo and protonated species in the aqueous medium and/or complications posed by the concerned bioligands, the shape of formation curves,in some lead-bioligand systems, is quite deviated from the expected sigmoidal shape. However, in other cases, there is a good resemblance between the statistical and the graphical log K_s values. Further, the involvement of kinetic factor cannot be ruled out. The metal bioligand interaction is rapid and no time lag exists at attaining equilibrium, as indicated by a sudden change in pH on the addition of alkali-metal hydroxide solution to the solution of Pb (II) salt, the bioligands and the mixture of Pb (II) and bioligand.

Table I: Computed values of log K_s at 20^0C for Pb (II) - Bioligand complexes.

S.No	Bioligands	pka	$logk_1$	$logk_2$	$logK_s$ (C*)	$logK_s$ (G*)
1	Thiobarbituric Acid	12.81	9.58	5.93	15.51	15.51
2	Barbituric acid	12.5	8.47	4.78	13.25	13.26
3	Folic Acid	8.26	3.27	0.59	3.86	3.76
4	Glutathione	9.65	6.79	2.12	8.91	8.88
5	Imidazole	7.00	1.75	0.00	1.75	1.30
6	Benzimidazole	5.53	0.72	0.00	0.72	0.57
7	Methyl-Imidazole	7.45	1.76	0.00	1.76	1.53
8	Dopamine	10.6	6.11	2.90	9.01	8.62

C*=Calculated values, G*=Graphical values

Fig. 1-8

Fig.1Pb (II)-ImidazoleFig.2Pb (II)-BenzimidazoleFig.3Pb (II)-Methylimidazole

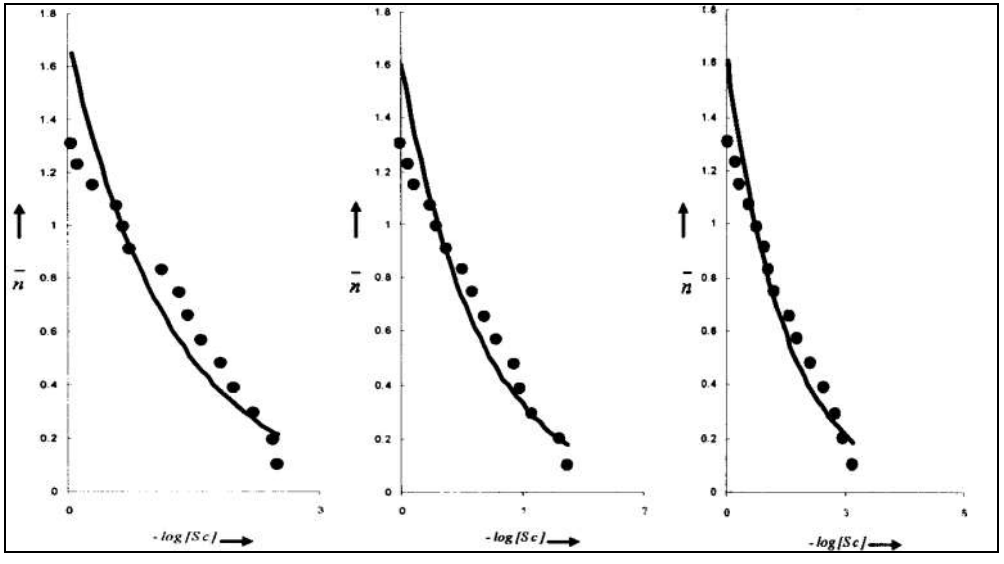
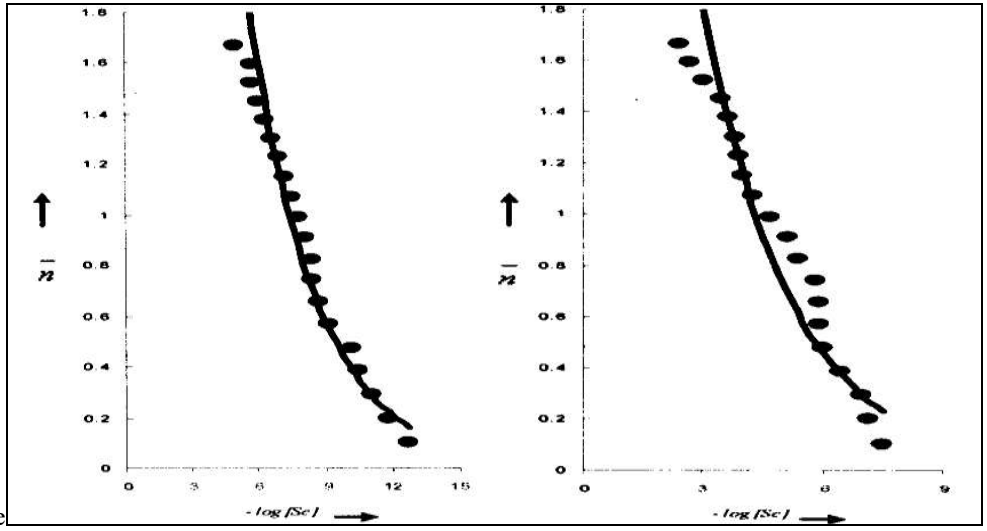


Fig.4 Pb(Il)-Thiobarbituric Acid Fig.5: Pb(lI) –



Dopamine

Fig.6 Pb(II)-Barbituric AcidFig.7 Pb(II)-Folic acid

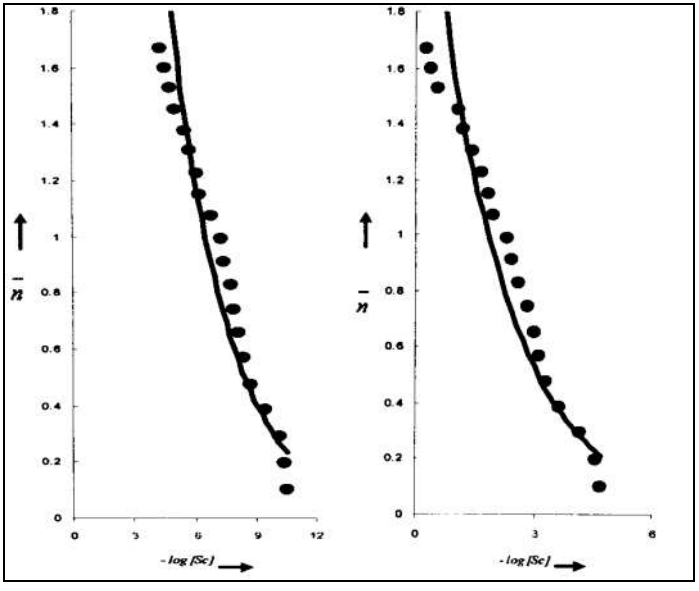
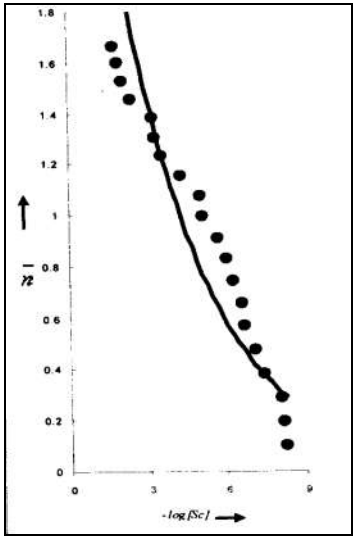


Fig.8 Pb (II)-Glutathione



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