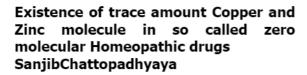
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ABSTRACT

Atomic Absorption Spectrophotometry is a very sensitive method, especially for detecting copper and zinc. Very minute amount of those metals were found in five potencies (6c, 30c, 200c, 1000c, and commercially available 10000c) of homeopathic medicine Cuprum metallicum and Zincum metallicum by applying the said method though the last four potencies were beyond Avogadro's limit, where no molecule of the original medicine can theoretically exist. However, no significant trace of these metals was found in the same potencies of placebo, prepared by the same pharmacy. Several other workers have also obtained the same result. It can be suggested that by serial succussion and centesimal dilution with alcohol the solute molecules become more tightly covered by solvent molecules with the increase of potency. As a result

dielectric constant of the solution falls, and non-homogeneity becomes established. Whenever one tries to prepare the next potency, the solute molecules by virtue of their enormous speed than the solvent molecules enters into next dilution like gaseous molecules, but unlike gases they cannot return to the original vial due to loss of homogeneity. With the increase of dilution such tendency increases, i.e., more proportion of solute molecules become transferred to the next serial dilution without obeying the simple law of dilution.

Key Words: serial dilution, atomic absorption spectrophotometry, zero-molecular dilution, non-homogeneity.

INTRODUCTION

The scientific community contravenes the basic principle of homeopathy, because it is the infinitesimally diluted medicines where no medicine molecule is said to be left. It can be mathematically proved that all themolecules of the original remedy become diluted away after reaching 12th centesimal dilution (12c). Hence, the homeopathic remedies of 30c, 200c, 1000c or 10000c, sold in the markets are said not to have any curative power. Benveniste was a French scientist who upraised the debate by claiming the controversial effect of zero —







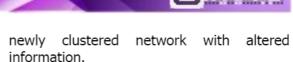


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molecular aqueous dilution of anti- IgE molecules on staining ability of basophil granules (Davenas et al, 1988). This was very much similar to zero-molecular dilution of homeopathic drugs. There are several theories to explain the effect of zeromolecular dilution of homeopathy. Among them 'Molecular Imprint Model' (Hadji et al,1991), 'Crystal Water Model' (Chaplin, 2000, 2007; Pan et al 2003; Sukul et al, 2005), and 'Contonian Model' (Young, 1975; Berliocchiand Conte, 1994; Conte et al, 1994) are significant. All of them assert that some special features (physical or chemical) develop in the solvent with the diluting away of the solvent molecule in the form of altering electromagnetic sianal, crystallographic structure of water, or even highly energizedspace-time character (singularity), so that the solvent becomes 'healthy water' itself that can work as medicine. Although they can neither reasonably explain the cause of appearance of 'proving symptoms' in healthy persons after applying such 'tuned solvent' or 'healthy water' at higher dose, nor can justify the cause of superiority of higher potencies over the lower ones. The model which describes alteration of crystallographic structure of water homeopathic drugs is not persuasive, because if we assume that water does store and transmit information of concerning solutes by means of its hydrogenbondednetwork, it would not work even, because, solutes of the body fluid may automatically re-equilibrate them and form



То prove the crystal water hypothesis true, several workers have described the alteration of hydrogen bonded network in homeopathic drugs by Fourier Transform Infra-Red spectra (Sukul et al, 2005). These hydrogen bonded networks are so specific for each medicine that they can be differentiated from each othereven at 'zero molecular state'. Hence it appears to us that the said medicine molecules might remain hidden impregnated within the solvent molecule, which is inseparable by any physical, chemical or biological process, or nondetectable by an ordinary spectrophotometer, except if we produce flame of the solute and inspect it with a highly sensitive spectrophotometer. Rao et al (2007) obtained preliminary data for the existence of molecules using Raman Ultra-Violet-Visible (UV-Vis) spectroscopy that can illustrate the ability to distinguish twodifferent homeopathic medicines (Nux vomica and Natrum muriaticum) from one another and to differentiate, within a given medicine, the 6c, 12c, and 30c potencies.

MATERIALS AND METHODS

Commercially available four potencies of Cuprum metallicum(Cup-Met) and Zincum metallicum (Zinc met) homeopathic medicine (HAPCO) of dilution 6c, 30c, 200c, 1000c, and 10000c were selected for experimental study. This is because Cu and









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Zn are very sensitive materials for atomic absorption spectrophotometer (AAS); these medicines were subjected to AAS study (Perkin-Elmer AAS, Model EI2373, in the Department of Metallurgy, Jadavpur University) with 324.8 and 213.9 nm wavelengths, respectively in air-acetylene flame. Placebo-control samples of the same potencies were also subjected to AAS.

RESULTS

From the commercially available samples it was found that gradually decreasing but highly variable quantities of Cu and Zn are present in homeopathic dilutions, but no detectable results were found in placebo. Higher quantity of Cu was found in 6c potency, which gradually but non-linearly decreased with dilution, even in 10000c dilution there were traces of Cu or Zn.

Table 1 Presence of Cu and Zn in Cup-Met and Zin-Met in ppm (Mean +SD)

ppin (wear ±30)		
Potency	Cu	Zn
	0.1 ±0.01	0.06 ±0.005
6c		
	0.05 ±0.01	0.04 ±0.005
30c		
	0.04 ±0.005	0.02 ±0.003
200c		
	0.04 ±0.006	0.01 ±0.002
1000c		
	0.03 ±0.006	0.01 ±0.002
10000c		

DISCUSSION

There are some theories, which focus on entrapped drug molecules (Chattopadhyay, 2002, 2003, 2006, 2016) of different nanoparticles (Chikramane et al, 2010, 2012; Ives et al, 2010; Bell et al, 2013), are quite different from other theories, as mentioned earlier. They state that diluting away of molecules is possible so long as the solution remains homogeneous. Homogeneity is gradually lost with the progress of potencies, so that ideal dilution of molecules is not possible by Hahnemannian or Korsakovian method of dilution. Traces of medicinal molecules remain at the (Chattopadhyay, 2002, 2003, 2006, 2016) or levitate at the top layer of the solution (Chikramane et al, 2010, 2012), from where they move to the next potency, so that real dilution of the solution does not occur if ritualized succussions are made between each two steps of serial dilutions. In other words, the process of diluting away of molecules becomes slackened due to ritualized succession. Moreover, the addition of ethanol with the solvent intensifies the slackening process (Chattopadhyay, 2002, 2003)to a great extent. Hence, reaching of 'zero-molecular state' becomes delayed infinitely. According to Rao et al (2007) succussion might also be responsible for creating very tiny bubbles (nano bubbles) that could contain gaseous inclusions of oxygen, nitrogen, carbon dioxideand possibly the homeopathic source material. We have seen that after each succussion, numerous tiny bubbles emerge









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from the bottom of the container and the solution becomes frothy for a short period, indicating highly organized state of ethanol molecules thandistilled water. molecules might be oriented around the hydrated drug moleculeforming one or more concentric inverted layers. Characteristic changes also appear in NMR spectra (Young, 1975). Existence of a metallic element can directly be proved by atomic absorption spectrophotometer better than other means, because each metal can produce flame of specific color when burnt, which is not dependent upon any type of ionic covalent or hydrogen bonding. Using atomic spectroscopy, Chikramane et al (2010, 2012) found that there are nanogram quantities of the starting material still present in these 'high potency' remedies preparedfrom metallic salts (Zincum met, Stannum met, Cuprum met, Argentum met, Aurum met, and Platinum met), even after the 200c dilution form of in nanoparticles. According to them traces of 'nanoparticles of the starting materials' levitate (float) in thetop 1% 'upper layers' of the solution, which is transferred to new vial for making higherpotencies. Later they showed that once the bulk concentration is below a threshold level of a few nanograms per milliliter, at the end of each dilution step, all of the nanoparticles levitate to the surface and are accommodated as a monolayer at the top. This dominant population at the air-liquid interface is preserved and carried to the subsequentstep, thereby formina an

asymptotic concentration. It means that that the concentrations of these starting materials, albeit at extremely low (picogram/milliliter) levels, did not decrease as expected with serial dilutions but instead formed an asymptote beyond the 6c potency (dilution factor of 1012), which directly corroborates with the prediction of Chattopadhyay (2003). Chikramane et al (2010, 2012) thus found that homeopathic medicines preparedusing metal powders as the starting raw materials retained them even at extremepotencies of 30c and 200c (dilution factors of 10⁶⁰ and 10⁴⁰⁰, respectively). In spite of such enormous dilutions, nano-particles of the metals ranging in sizes from 5 to 10nm were detected bν transmission electron microscopy (TEM) and electron diffraction (ED). There might be no unanticipated finding of Chikramane et al (2010, 2012), and eventually supported by several workers (Ives et al, 2010; Bell et al, 2013), but some difficulties can be pointed out for the hypothesis included therein. The question arises if the drop is pipetted out from the middle or bottom layer? It may be solved by the following way: the original substance initially remains more concentrated towards the bottom of the solution, though whenever one tries to transfer, it moves to the fluvialpart of the solution or surface like that of a gaseous substance and towards reaching acritical value its concentration becomes asymptotic to dilution (Chattopadhyay, 2003, 2016), the similar result has been obtained in the









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present work. The main thing is theloss of homogeneity, and it hardly matters from where one is pipetting the liquid, fromthe top, middle or bottom of the vial. Even if Korsakovian method is applied the drug molecules at a very high dilution strike the wall of the container with enormous velocity and remain stuck up there. Thus they remain in the subsequent dilution prepared from the same vial and thus loss of homogeneity cannot be prevented. The method for precipitation of proteins from theiraqueous solution by the gradual addition of cold ethanol, as derived by Cohn and Edsallin 1943, is a good example of secondary attainment of non-homogeneity. The positive and negative charges of protein molecules remain separated widely in aqueous mediumdue to high dielectric constant of water. By the gradual addition of ethanol having low dielectric constant the attraction between opposite charges within protein molecule increases and they aggregate and precipitate. The concept is true for not only proteins butfor all charged molecules, having high molecular weight. molecules Hydrated charged concentrated at the bottom region of the ethanol solution and if the bound wateris removed, the charged molecules become precipitated, nicely implemented in DNA and isolation technique from solutions. For low molecular substances, or those who are more soluble in ethanol than water, this event does not occur prominently, but there is always a tendency of precipitation. Samal and Geckeler(2001),

while investigating with full erene molecules were found clumping together, first as clusters of molecules, then as bigger aggregates of those clusters when diluting in aqueous solution. Far from drifting apart from their neighbors in course of dilution, they got closer together. It may be cited as an example of fall of dielectric constant with progressively increasing of dilution. Initially, at the beginning of dilution, the adjacent water molecules remain wide apart in a low diluted solution, sparsely covering the surface of the solute molecule. Conversely, when the solution reaches to ultra-high dilution, succussions can bring water molecules closer, tightly covering the entire surface of solute molecules, there is a loss of homogeneity (Chattopadhyay, 2003) and therefore, fluvial transfer of drug molecules increases. The workers Samal and Geckeler (2001), thus found that the football ('buckyball') like molecules kept forming by untidy aggregates in the bottom of the solution with the increase of serial dilution. When they did the same experiments with cyclodextrin they behaved the same wav. the organic So molecule sodiumquanosine monophosphate, DNA and plain old sodium chloride. Hence, it may bepresumed that the solute molecules generally becomes aggregated in the bottom of thecontainer, though there may be exceptions, but whenever one tries to transfer any volume to prepare the next dilution the solutes rush to the fresh vial in much higher velocity thanthe solvent molecules, but cannot return to the original







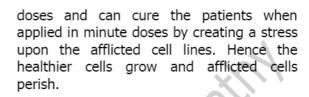


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vial due to loss of homogeneity. With the increase of dilution such tendency increases, i.e., more proportion of solute molecules become transferred to the next serial dilution without obeying the simple lawof dilution. Lastly, a theoretical condition may arise when the entire solute molecule become restricted in 1% volume of the diluted drug, which corresponds the concept of critical dilution (Chattopadhyay, 2003, 2016). Hence, the available experimental study Chikramane et al (2010, 2012)as well as the present did not manifest any further significant fall of metallic concentration with serial dilution. According to some workers (Sukul et al, 2005; Chattopadhyay, 2002, 2016) potentization by succussion is comparable to sonication, causing special orientation of ethanol molecules or water. Hence, altered orientation of hydrogen bonded network is therefore not the cause of potentization, but the effect of the same. It forms inverted micelle like orientation around hydrated drug molecules, even may form ethanol bilayers like that of lipid bilayers as seen in drug loaded liposomes (Chattopadhyay, 2002),like nanobubble or nanoparticle of (Chikramane et al (2010, 2012). With the increase of potency the strength of ethanol capsule increases as it becomes smaller and compact, causing penetration of the same far interior tothe cell through the plasma membrane producing influence upon minute controlling protein factors interior to the cells. Thus it possibly can arouse physiological symptoms inhealthy individuals when applied in higher



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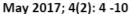


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