Interaction Studies on Vitamin K (Menadione) and Its Nano Aggregates in Aqueous Solution and Mixed Aqueous Solution using UV-visible Spectroscopy

Vibhuti Gugnani, Harpreet Singh

Department of Chemistry, Lovely Professional University, Phagwara-144 4111,

Abstract

Interactions between vitamin K (menadione) and different electrolytes present in plasma (sodium chloride, potassium chloride, magnesium chloride and calcium chloride) have been carried out in aqueous solutions using UV-visible spectroscopy. further the nanoaggregates of menadione have been synthesised and interactions study of these nanoaggregates with said electrolytes have been carried out. On comparison of both, it has been found that philic kind of interactions between nanoaggregartes becomes more prominent as compared to vitamin K compound.

Introductions

Any foreign agent (drug, vitamin and other supplements) in any dosage form when introduced into the living system has to interact with different molecules; electrolytes, amino acids, proteins, sugar, nucleosides, nucleotides etc. in aqueous solutions at different concentration and pH. In order to store these pharmaceutical formulations to have an appropriate shelf life, the study on the physicochemical parameters of the active component of these formulations at different temperatures *in vitro* is also of great importance and great value. Also many times more than one drug is co-administered into living system and different excepients/preservatives are the integral part of any formulation. The body fluid/cytoplasm/extracellular tissue is well defined solution of many small electrolytes and biomolecules and these molecules play pivotal role for transfer and transport to the target site to show their effect. Vitamin K is a fat soluble vitamin and having a significant role reform of proteins required in blood coagulation and bone metabolism [1,2]. Vitamin K1(phylloquinone) and the vitamin K2 (menaquinones) are naturally occurring classes of vitamin K. Vitamin K3 (menadione) is a synthetic substitute of vitamin K, can be supplemented in animal feeds. 2methyl-1,4-naphthoquinone moiety is present commonly in all kinds od vitamin K, but varies in length and saturation of aliphatic chain. [3] Vitamin K has role in functioning of brain such as; pathogenesis of Alzheimer's and Parkinson's Disease, development and aging, brain sulfotransferase activity, mitochondrial electron transfer etc. [4,5] It can prevent oligodendrocytes and neuron cell death due stress. Many biological and physiological processes witness the presence of members of vitamin K family. [8]

Menadione (vitamin K3) is a synthetic pro-vitamin having quinone group which seems to be effective against inflammation and haemorrhage [9] Also it is stable and inexpensive than natural counterpart. [10] Menadione along with its derivatives is effective against the cancer cell lines of breast, colon and gliomas.

[11-16] Few reports also shown it as potent antitumor agent against some rodent and human tumor cells *in vitro*. [17]

The study of processes occurring in solution phase are quite relevant from medical and pharmaceutical angle. Among them solvophilic-solvophobic interactions in solutions, solubility and stability of the drug/vitamin are prominent issues understand the fate of these molecules in biological media. On the basis of these objectives, the available literature has been reviewed to form the foundation of present study.

From the available reports, it is clear that data is still lacking on the interactions study of menadione in aqueous solution of electrolytes (sodium chloride, potassium chloride, magnesium chloride and calcium chloride). Therefore, in the present study has been planned to carry out the drug-electrolyte interactions at temperature 298K and atmospheric pressure keeping pH neutral. Since vitamin K is fat-soluble, therefore, nanoaggregates of vitamin K are synthesized to increase the solubility in aqueous medium. The synthesis of organic nanoaggregates (ONAs) of menadione will be carried out and its interactions of ONAs of menadione with different electrolytes. The main objectives of the present research work are interactions of menadione drug with different electrolytes in aqueous solution at neutral pH., synthesis of organic nanoaggregates (ONAs) menadione and interactions of ONAs of menadione with different electrolytes in aqueous solution at neutral pH. Attempt will be made to compare the interactions shown by menadione and ONAs of menadione in aqueous phase.

EXPERIMENTAL

The materials used the present study are; menadione (vitamin K), sodium Chloride, magnesium chloride, potassium chloride and calcium chloride. All the chemicals used are of AR grade and are procured from Loba Chemie India and are used without further purification.

Double distilled water have been used to make solutions. All the solutions were freshly prepared. Digital balance having precision up to 10^{-3} mg have been used for weighing purposes.

UV-VIS spectroscopy is the methodology used for this experiment to record the absorbance spectra of the interactions between different electrolytes and micro-molar solution of vitamin K and its nanoaggregates. UV 1800 Shimadzu is the instrument used for analysis of this study. UV-VIS Spectroscopy is based on Beer-Lambert law. According to which, when light is passed through a sample solution, the quantity of light absorbed by it is directly proportional to the concentration of solute present in it and the path length of the light through the sample solution.

RESULT AND DISCUSSION

In the present study, the physicochemical interactions of different electrolytes with menadione drug at micro molar level and at nano scale in aqueous solutions have been carried out taking 3 different concentrations using UV- Visible spectroscopy.

Interaction of Vitamin K with different electrolytes

As is clear from figure 1. At neutral pH, menadione show three absorption maxima at ≈ 330, 250 and 220 nm. When sodium chloride solution is added into this solution, the adsorption maxima start decreasing at wavelength 330 and 250 nm and diminish at 220 nm. On further addition of sodium chloride, absorption maxima further flatten at 330 nm and tend towards same direction at wavelength 250 nm.

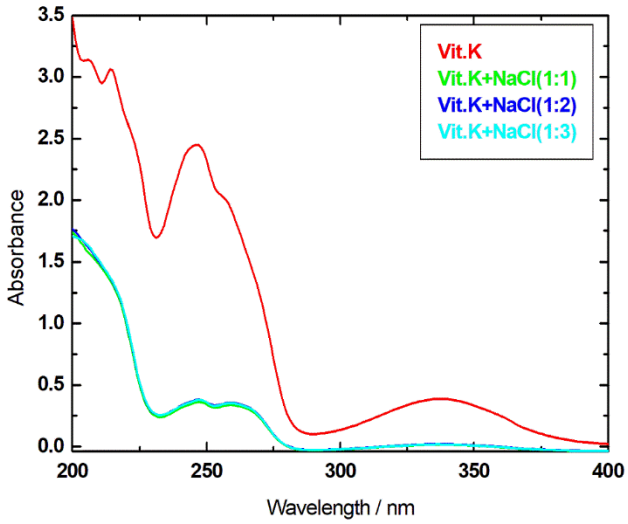

Figure 1. Absorption spectra of interaction of vitamin K with NaCl at different concentrations

The possible interactions which may occur in this system are: a) Hydrophilic-ionic interactions between anions and cations of electrolytes and electron pairs of the oxygen atom of the menadione. (Figure 2.) b) Hydrophobic–ionic interactions between aromatic part of menadione and cations and anions of the electrolytes.

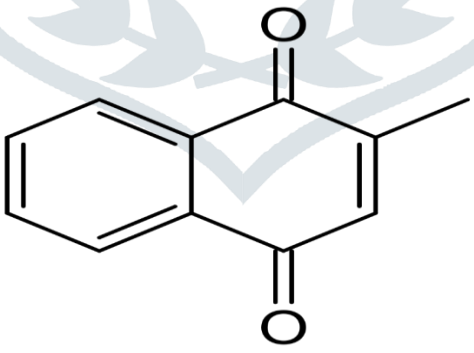

Figure 2. Structure of Menadione

The philic interactions enhance the absorption maxima intensity and the phobic interactions diminish the absorption maxima intensity. In the present study, phobic interactions seem to be playing a significant role and diminishing of absorption maxima has been observed (Figure 3.) which is also in line with the literature data that hydrophobic menadione interacts very less at neutral ph in aqueous medium.

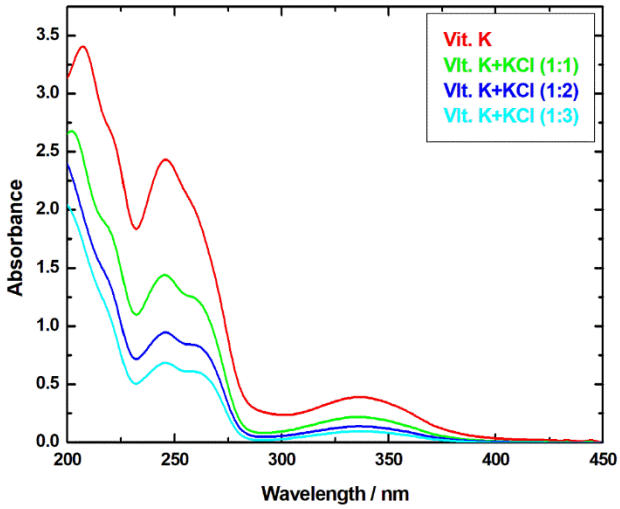

Figure 3. Absorption spectra of interaction of vitamin K with KCl at different concentrations

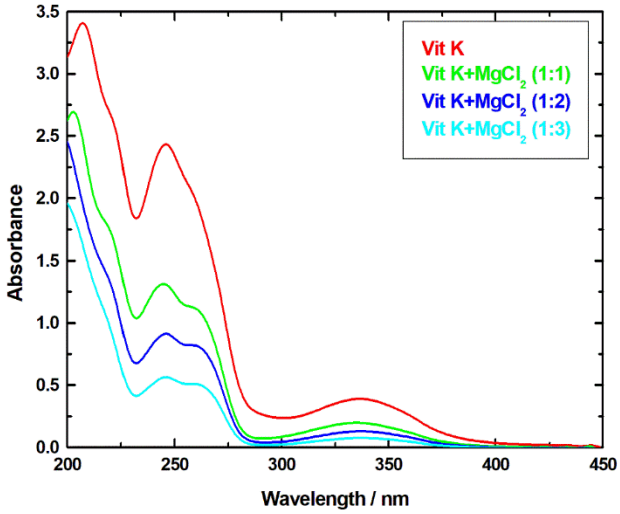

Figure 4. Absorption spectra of interaction of vitamin K with $MgCl_2$ at different concentrations

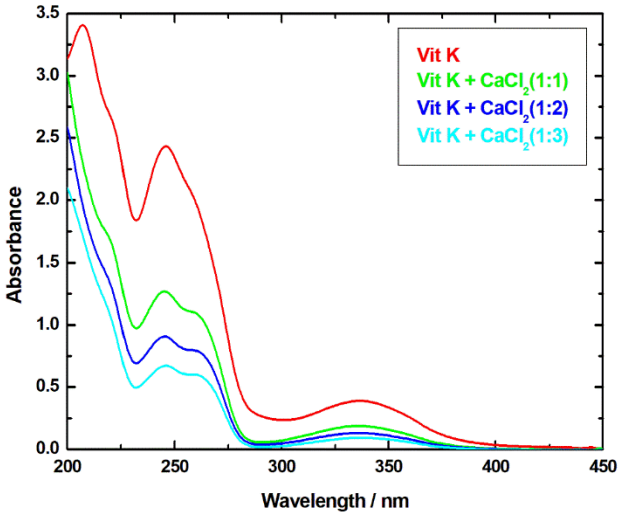

Figure 5. Absorption spectra of interaction of vitamin K with $CaCl_2$ at different concentrations

Similar trends of decreasing absorption maxima at all the three wavelengths have been observed in the studied electrolytes solutions i.e. potassium chloride, magnesium chloride and calcium chloride (Figure 3-5) which further decreases with addition of respective electrolytes.

Interaction of Vitamin K and Nanoaggregates of Vitamin K

Many compounds at nano level, show very different properties. The interactions of menadione and electrolytes have further been explored at nano levels by synthesizing the organic nanoaggregates (ONAs) of menadione. Figure 6 shows the conversion of menadione to organic nanoaggregates clearly which needs further confirmation from advanced techniques like fluorescence, DLS, SEM and TEM.

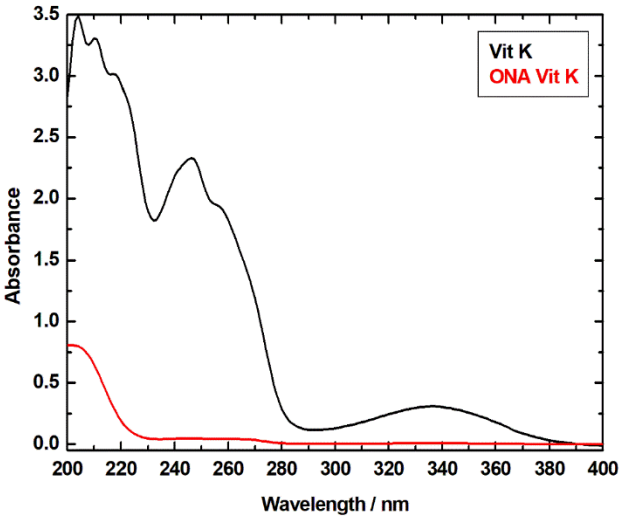

Figure 6. Absorption spectra of vitamin K with its organic nanoaggregates (ONAs)

Effect of dilution on Nanoaggregates

The effect of dilution has been observed on the organic nanoaggregates(ONAs) of vitamins K (menadione) as shown in figure in 7. As is clear from the figure that the ONAs are quite stable at different dilutions but the decrease in absorption maximum have been observed with concentration.

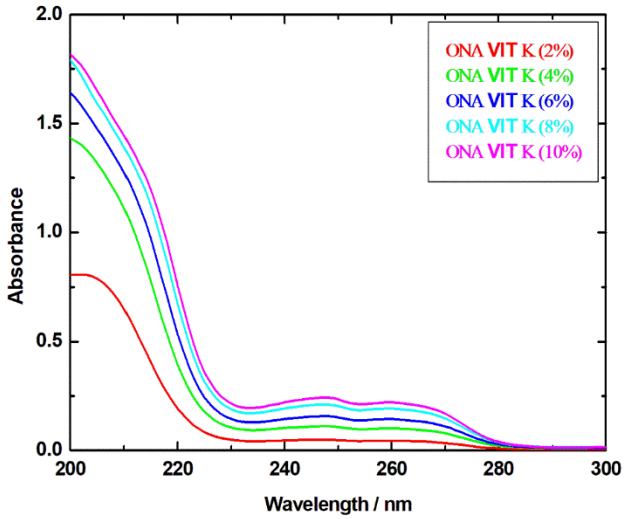

Figure 7. Absorption spectra of effect of dilution on nanoaggregates

Interaction of Nanoaggregates of vitamin K with various electrolytes

As far as the interactions with different electrolytes are concerned, the ONAs of menadione show similar trends as observed in case of menadione drug for all the electrolytes i.e. the absorption maxima decrease on the addition of aqueous electrolyte solutions which further diminish with concentration of electrolytes. (Figure 8-11)

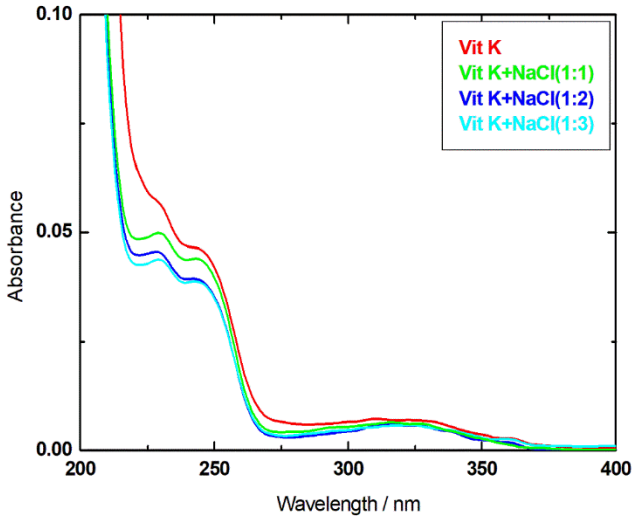

Figure 8. Absorption spectra of ONAs of vitamin K and sodium chloride at different concentrations

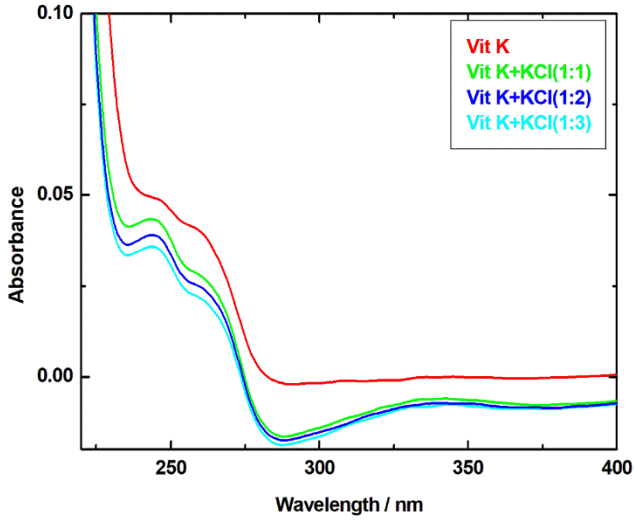

Figure 9. Absorption spectra of ONAs of vitamin K with potassium chloride at different concentrations

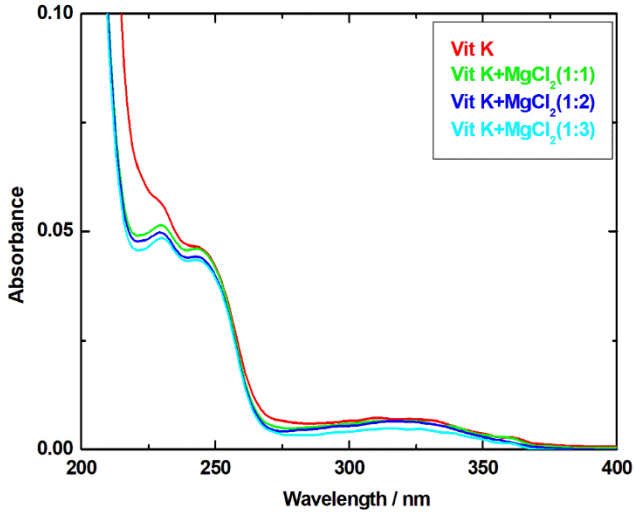

Figure 10. Absorption spectra of ONAs of vitamin K and magnesium chloride at different concentrations

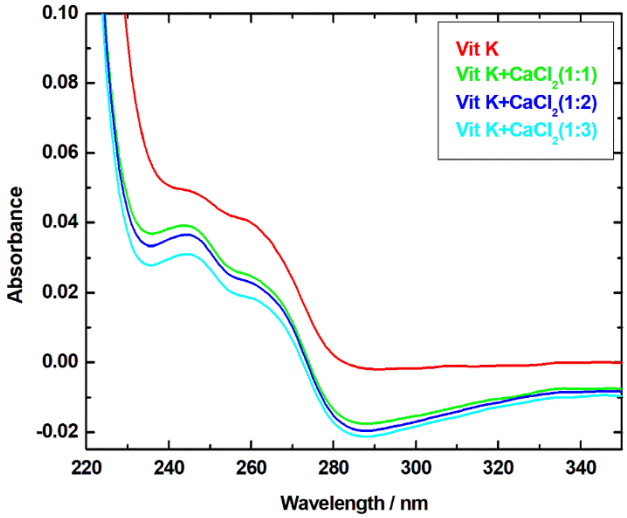

Figure 11. Absorption spectra of ONAs of vitamin K and calcium chloride at different concentrations

CONCLUSIONS

Interactions between vitamin K (menadione) and its organic nanoaggregates (ONAs) and different electrolytes present in plasma (sodium chloride, potassium chloride, magnesium chloride and calcium chloride) have been Studied in aqueous solutions using UV-visible spectroscopy. it has been found that philic kind of interactions between nanoaggregartes becomes more prominent as compared to vitamin K compound. Further characterisation and interactions of ONAs with different biomolecules need the confirmation form other sophisticated instrumental techniques to comment and confirmation of the present observations.

References

1. J. W. Suttie, Mechanism of action of vitamin K: synthesis of gamma-carboxyglutamic acid, CRC Crit. Rev. Biochem. 1980, 8, 191− 223.
2. P. A. Price, Role of vitamin-K-dependent proteins in bone metabolism. Annu. Rev. Nutr. 1988, 8, 565−583
3. M. J. Shearer, A. Bach, M. Kohlmeier, Chemistry, nutritional sources, tissue distribution and metabolism of vitamin K with special reference to bone health, J. Nutr. 1996, 126, 1181S−1186S.
4. T. D. Bjornsson, In Effects of Drugs on Nutrition; Roe, D. A., Campbell, T. C., Eds.; Marcel Dekker: New York, 1982; 114.
5. C. Cannes, F. Kanoufi, A. J. Bard, Cyclic Voltammetric and Scanning electrochemical microscopic study of menadione permeability through a self-assembled monolayer on a gold electrode, Langmuir 2002, 18, 8134-8141
6. M. Kaneki, T. Hosoi, Y. Ouchi, H. Orimo, Pleiotropic actions of vitamin K: protector of bone health and beyond?, Nutrition. 2006, 22, 845–852.
7. M.S. Elgawish, C. Shimomai, N. Kishikawa, K. Ohyama, M. Wada,N. Kuroda, Development and validation of the first assay method coupling liquid chromatography with chemiluminescence for

the simultaneous determination of menadione and its thioether conjugates in rat plasma, Chemical research in toxicology, 2013, 26, 1409-1417.

8. D. Ivanova, Z. Zhelev, P. Getsov, B. Nikolova, I. Aoki, T. Higashi, R. Bakalova, Vitamin K: Redox-modulation, prevention of mitochondrial dysfunction and anticancer effect, Redox Bio., 2018, 16, 352-358.
9. J. Verrax, J. Cadrobbi, C. Marques, H. Taper, Y. Habraken, J. Piette, P. B. Calderon, Ascorbate potentiates the cytotoxicity of menadione leading to an oxidative stress that kills cancer cells by a non-apoptotic caspase-3 independent form of cell death, Apoptosis, 2004, 9, 223-233.
10. M.F. Vita, N. Nagachar, D. Avramidis, Z.M. Delwar, M.H. Cruz, A. Siden, K.M. Paulsson, J.S. Yakisich, Painkiller effect of prolonged exposure to menadione on glioma cells: potentiation by vitamin C, Invest. New Drugs, 201, 129,1314-1320.
11. M. Redaelli, C. Mucignat-Caretta, A.A. Isse, A. Gennaro, R. Pezzani, R. Pasquale, V. Pavan, M. Crisma, G. Ribaudo, G. Zagotto, "New naphthoquinone derivatives against glioma cells", Eur. J. Med. Chem. 2015, 96, 458-466.
12. D.T. Breslin, J.E. Coury, J.R. Anderson, L. McFail-Isom, Y. Kan, L.D. Williams, L.A. Bottomley, G.B. Schuster, Anthraquinone photonuclease structure determines its mode of binding to DNA and the cleavage chemistry observed, J. Am. Chem. Soc., 1997, 119, 5043-5044.
13. D.W. Lamson, S.M. Plaza, The anticancer effects of vitamin K**,** Altern. Med. Rev. 2003, 8, 303-318.
14. D. Bhasin, S.N. Chettiar, J.P. Etter, M. Mok, P-K Li, Anticancer activity and SAR studies of substituted 1,4-naphthoquinones, Bioorg. Med. Chem., 2013, 21, 4662-4669.
15. M. Cioroi, C. Georgescu,D. Tutunaru, The quantification of menadione as nutritional supplement in feedingstuffs, Romanian Biotechnological Letters, 2015, 20 , 10431-10438.
16. F. D. Miloglu, Y. Kadioglu, O. Senol, GC-FID and HPLC-DAD Methods for the determination of menadione sodium bisulphite directly and by converting menadione sodium bisulphite to menadione in pharmaceutical preparation, Iranian journal of pharmaceutical research: IJPR. 2014, 13, 353.
17. M. L. Toole, P. S. Douglas, R. H. Laird, D. B. Hiller, Fluid and electrolyte status in athletes receiving medical care at an ultradistance triathlon, Clin J Sport Med., 1995, 5, 116-22.