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Interaction of Serum Proteins In Evaluating the Efficacy of Sevelamer Hydrochloride and Sevelamer Carbonate Together with Dietary Sources In Hyperphosphatemia Condition

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ABSTRACT

Kidney is one of the vital organs in the human body. The function of the kidney involves purification of the blood, reabsorption of water, excretion of waste, secretion of hormones like adrenaline. When the kidney becomes non-functional, it leads to a condition called chronic kidney disease (CKD), where the kidney fails to purify the blood. When this happens, the patients are expected to switch over to artificial methods of purifying the blood such as dialysis. Patients suffering from CKD have high levels of phosphate (hyperphosphatemia) and leads to calcification of blood vessels. In order to reduce phosphate levels the patients are recommended drugs which act as phosphate binders like sevelamer hydrochloride and sevelamer carbonate. In the present investigation the banding patterns of serum proteins and the serum along with plant samples and the drug were analysed through Native –PAGE. The serum protein interaction studies showed a positive result for sevelamer hydrochloride where as the sevelamer carbonate displayed less interaction. The swelling property of the drug was studied using phase contrast microscopy. It was observed that the drug swells up when treated with water and also the content of the drug slowly dissolves with time.

Keywords: CKD, hyperphosphatemia, calcification, phosphate binders.

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INTRODUCTION

Sevelamer is a phosphate binder that is given as a medication to treat hyperphosphatemia in patients suffering from chronic kidney disorder. It binds to the dietary proteins and prevents the absorption of the same in the body. It is mainly marketed in two forms- hydrochloride form and carbonate form. Genzyme developed sevelamer which acts as a phosphate binder that contributes to the treatment of hyperphposphatamia in CKD. Thus, is classified pharmacologically as a phosphate binder (CHMP assessment report, 2009). Sevelamer carbonate structurally consists of cross linked polymer of allylamine carbonate. Epichlorohydrin serves as a cross linking agent. The two secondary amines in the structure are obtained from cross linking agents, polyallylamine hydrochloride (starting material) and a molecule of epichlorohydrin. (CHMP assessment report, 2009).

The uplifting news is super nourishments contain cell reinforcements that help kill free radicals. Indeed, even in generally low sums, cancer prevention agents can help moderate or stop the rate of oxidation brought on by free radicals. Cases of cell reinforcements incorporate flavonoids, lycopene and vitamins C, E and beta-carotene.

The present work is aimed to contribute to the same theory and also show the effect of the samples chosen on the drug efficiency. The sample chosen are bitter gourd, plantain stem, squash (chow chow), watermelon seeds (dry), sunflower seeds and flax seeds. These samples are chosen on the criteria that it is easily available and also is known as the home remedy diet for a person suffering from CKD. It is mandatory to make sure that the food such patients consume is less in potassium, sodium, fluids etc.

MATERIALS AND METHOD

Plant source:

Plantain stem (*Musa paradisiaca),* Bitter gourd (*Momordica carantia),* Chow-Chow (*Sechium edule),* Flax seeds (*Linum usitatissimum),* Sunflower seeds (*Helianthus annus),* Water melon seeds (*Citrullus lanatus).* These were obtained from Nilgiri's Supermarket, Bengaluru, Karnataka, India.

Drug source:

Sevelamer Hydrochloride and Sevelamer Carbonate was obtained from Balaji Medical store, Bengaluru, Karnataka, India.

Source of clinical samples:

Human blood serum:

5.0ml of the blood sample was collected and was added into a centrifuge tube. EDTA was added and centrifuged at 2000rpm for 5 mins in order to obtain serum.

Equipment:

The equipment used were UV-Vis spectrophotometer (Model no.117), Centrifuge (REMI laboratory, Maharashtra, India), Phase contrast microscope (Olympus, Japan. /Model no. Infinity-1 Lumenera), pH meter (ELICO Ltd., Hyderabad, India. / Model no. LI.120), Electrophoretic unit, Gel rocker (TARSONS Product Pvt Ltd., Kolkata, India. / Model no. CAT.4080), weighing balance (Shimadzu/ Model no. ELB300), hot plate (TARSONS Product Pvt Ltd.) and Microwave.

Chemicals:

Acetone, Methanol, Diethyl ether, Petroleum ether, Ethyl acetate, Benzene, Chloroform, Sodium hydroxide, Hydrochloric acid, Acrylamide, Destaining solution, Glacial acetic acid, Ammonium persulphate (Fisher Scientific India Pvt Ltd., Mumbai, India.), Sulphuric acid, Tris HCl, Agarose, N,N,N',N'- Tetramethylenediamine, Gel loading dye, Coomassie Brilliant Blue (HIMEDIA Laboratories Pvt Ltd., Gujarat, India.),

Solubility:

50mg of the 2 drugs, sevelamer hydrochloride and sevelamer carbonate were checked for their solubility in 5.0ml in distilled water, 5%HCL, 5%NaOH, Conc. H_2SO_4, acetone , ethanol, methanol, chloroform, benzene, diethyl ether, petroleum ether, ethyl acetate, phosphate buffer, bicarbonate buffer, acetate buffer, citrate buffer, Tris HCl.

Swelling:

A particle of sevelamer hydrochloride was placed on the cavity slide and this was observed under the Phase Contrast Microscope. An image of this was captured. This was followed by adding a drop of distilled water to the same to observe the swelling reaction of this particle. An image of this was captured. The same procedure was repeated for sevelamer carbonate particle.

Preparation of plant extract:

The 6 plant samples that were selected, for each of these samples organic and buffer extractions were prepared, resulting in 12 samples. For the organic extracts acetone, diethyl ether, methanol was used for preparing solutions of different concentrations for plant extracts of *viz*; 5%, 20%, 50%, resulting in 54 organic extractions of various concentrations. The 6 plant origin samples were used to prepare 2 buffer extracts using phosphate buffer and bicarbonate buffer of different concentrations *viz*: 5%, 20%, 50%, resulting in 36 buffer extractions. These extracts were further analysed for bioactive molecules using a UV- Vis spectrophotometer at different wavelengths.

Preparation of samples for native-page:

5.0ml of the 6 plant samples which were extracted in 0.1M phosphate buffer was centrifuged along with 0.2g of drug (sevelamer hydrochloride) and 1.0ml of serum. 5.0ml of the next set of plant samples extracted in 0.1M phosphate buffer was centrifuged, this time with 0.2g of drug (sevelamer carbonate) and 1.0ml serum. To analyse the above mentioned samples, 1.0ml of the human serum alone was centrifuged and 2 sets of 5.0ml of serum were centrifuged along with 0.2g sevelamer hydrochloride, 0.2g sevelamer carbonate which served as a standard.

Native-PAGE:

The samples were analysed for their banding patterns by NATIVE-PAGE according to Ornstein L and Davis, 1964.

RESULTS AND DISCUSSION

Chronic kidney disease (CKD) is one of the major problem faced in public health. Various types of therapies and drugs are administered to the patient suffering from end-stage renal disease such as renal replacement therapies. Patients undergoing dialysis can suffer from inaccurate mineral metabolism leading to high phosphate levels in the serum. The dietary intake of phosphate rich food is avoided during this condition as the kidneys are incapable of metabolizing them. To decrease the levels of phosphate a treatment involving phosphate binders were administered to the patients. These phosphate binders attach or bind to the high phosphate molecules/ ions present in the system and remove them efficiently from the body. This allows in the treatment of the hyperphosphatemia. Before, calcium binders were used to eliminate phosphate levels in the serum, but this led to a hypercalcemic condition. This can lead to the accumulation of calcium in the arteries of the heart which can lead to the blockage of it. Thus, sevelemer which is a non-calcium non- magnesium and aluminium free phosphate binder was made so that there is no calcification seen in the body nor there is metal toxicity in the blood serum.

Solubility:

Solubility is a phenomenon in which dissolution of the solute in a solvent which results in a homogenous solution.

Table 1: Showing the solubility of drug in different solvents.

Sl. No.	Solvent	Solubility
1.	Water	-
2.	5% HCl	-
3.	5% NaOH	-
4.	Conc. H_2SO_4	-
5.	Petroleum Ether	-
6.	Diethyl Ether	-
7.	Acetone	-

8.	Ethanol	-
9.	Methanol	-
10.	Chloroform	-
11.	Benzene	-
12.	Ethyl Acetate	-
13.	Phosphate buffer	-
14.	Citrate buffer	-
15.	Bicarbonate buffer	-
16.	Acetate buffer	-
17.	Tris HCl	-

The solubility of a solute depends on the solvent, temperature and pressure. Saturation concentration is the extent to which the solubility of a substance in a specific solvent. The solvent used is generally a liquid. The extent of solubility ranges from completely soluble to poorly soluble or insoluble. The two drugs, Sevelemer Carbonate and Sevelemer HCl were subjected to preliminary solubility test. Neither of them were found to be soluble in the solvents used for the study.

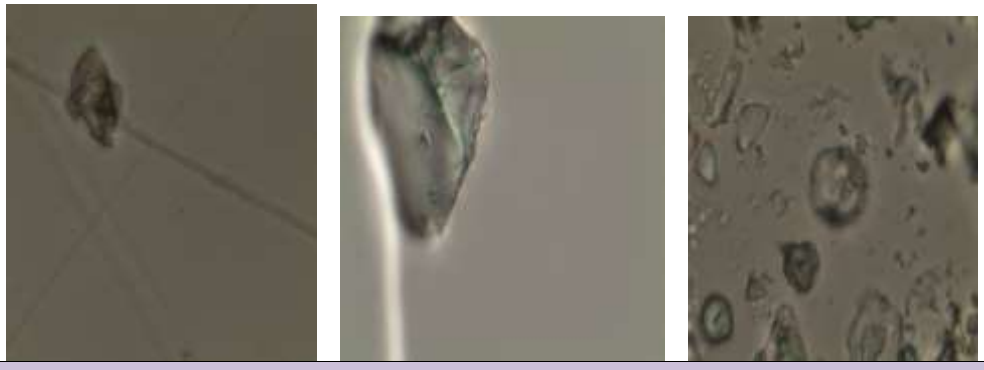
IUPAC defines solubility as the analytical composition of a saturated solution expressed as a proportion of a designated solute in a designated solvent. Solubility may be stated in units of concentration, molality, mole fraction, mole ratio, and other units.

The partition coefficient (Log P) is a measure of differential solubility of a compound in a hydrophobic solvent (octanol) and a hydrophilic solvent (water). The logarithm of these two values enables compounds to be ranked in terms of hydrophilicity (or hydrophobicity).

A drug to be absorbed should be present in the form of a solution at the site of absorption. Many techniques have been used to improve the solubility of the drug (poorly soluble) which involve chemical and physical modification of the drug and other methods like salt formation, solid dispersion, particle size reduction, use of surfactant, crystal engineering etc.

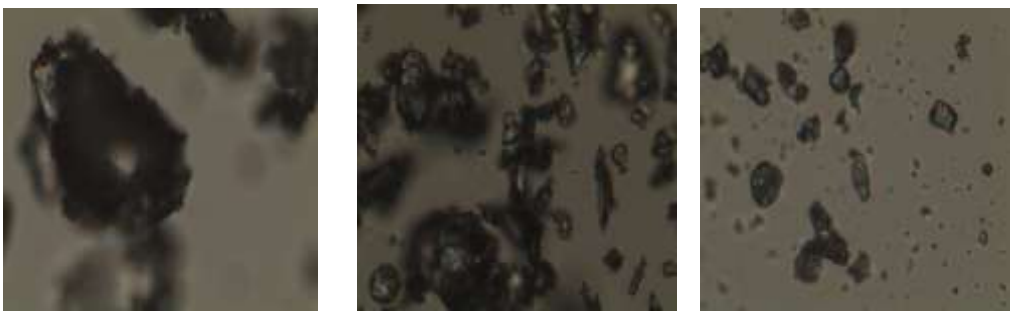
Swelling properties of the drug:

Sevelemer carbonate and Sevelemer hydrochloride shows property of swelling when treated with water. The results have been positive to this test.



(a) (b) (c)

Figure 1: Sevelemer carbonate under phase contrast microscope. (a) Sevelemer carbonate before swelling (b) Sevelemer carbonate after swelling.



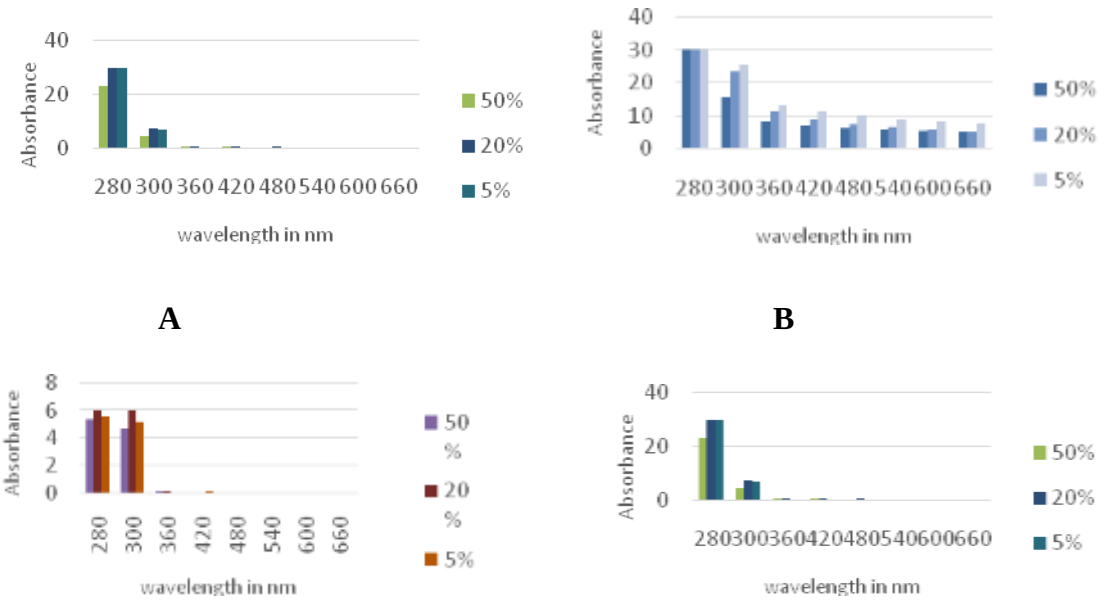
(a) (b) (c)

Figure 2: Sevelemer hydrochloride under phase contrast microscope. (a) Sevelemer hydrochloride before swelling (b) Sevelemer hydrochloride after swelling.

These results showed swelling range between 10 to 200% with an average being approximately 50-60%. The results were determined by phase contrast microscope 10X using Olympus, infinity1 lumenera. The results presented here will compare the experimental swelling measurements to that of the calculated values from the simulations. The molecular dynamic simulations are used to construct a view of the average structure of our model system in the presence of phosphate ions. It is further used to study simulation of the molecule.

Extraction of plant samples

The plant samples were extracted using organic and buffer extraction solvents and it was observed that acetone extractions gave the best result.



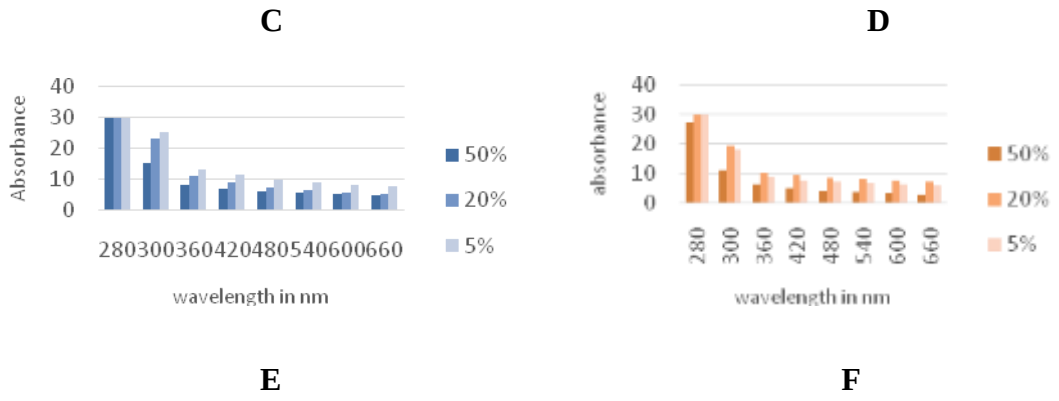


Figure 3: A- Acetone extraction of *Momordica charantia*, B- Acetone extraction of *Helianthus annus*, C- Acetone extraction of *Musa paradisica* D- Acetone extraction of *Momordica charantia*, E- Acetone extraction of *Helianthus annus*, F- Acetone extraction of *Linum ustatissimum* seeds

Figure 3 illustrates that 280nm absorbance of the plant samples gives the highest peak indicating the high amounts of proteins in them. Though acetone extraction was obtained the best, phosphate buffer extraction was carried out for further analysis as this experiment deals with the usage of an organ system.

For carrying out the different functions of the body, the body needs vitamins, minerals, lipids, carbohydrates, proteins and fiber which can be obtained from both animals and plants. Plant proteins contribute as food resource as they contain essential amino acids to meet the human physiological requirements. Proteins can be obtained from plants, animals and microorganisms. The major economical proteins can be obtained from plant from plant seed or legumes. The extraction of these natural proteins depends on the physicochemical properties of the proteins. The isolation and purification of a single protein (choice) from a mixture of proteins is achieved from the physical and chemical properties of the proteins. The following properties are characteristic to each protein: amino acid sequence, composition, size, net charge, heat stability, hydrophobicity, solubility. Based on these properties, several methods of extraction exist.

Interaction of drug with serum proteins:

Human serum was centrifuged with the two drugs and the samples taken into study. The drug values for each sample with serum after evaporation was found to be as given below in the table [Amount of drug taken = 0.2g]

Sevelemer carbonate along with serum and the samples values suggest that there has been a slight increase in the dry weight of these samples, except for watermelon seeds, showing that it could have interacted with certain proteins of the serum. This was confirmed by the bands that were seen

after Native PAGE was performed. Serum alone indicates 6 bands for the six proteins that is present in it but when the samples and serum was run with the drug displayed only few bands, not all 6, thus indicating there could have been interaction of the drug with some of the proteins such as Alpha 2 and Beta protein.

Table 2: Pellet weight of samples with Sevelemer carbonate and serum.

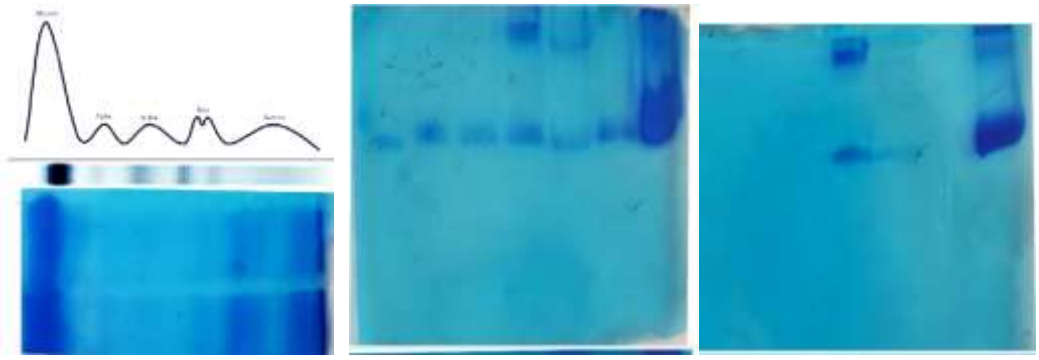
Sample name	Weight in g
Watermelon seeds	0.02
Flax seeds	0.39
Sunflower seeds	0.40
Bitter gourd	0.27
Chow chow	0.36
Plantain stem	0.29

Table 3: Pellet weight of samples with Sevelemer HCl and serum.

Sample name	Weight in g
Watermelon seeds	1.15
Flax seeds	0.50
Sunflower seeds	1.16
Bitter gourd	1.13
Chow chow	1.03
Plantain stem	1.64

Sevelemer HCl along with serum and the samples values suggest that there has been an increase in the dry weight of these samples even more than that seen with Sevelemer carbonate showing that it could have interacted with most proteins of the serum. This can be confirmed by the bands that were seen after Native PAGE was performed. Serum alone shows 6 bands for the six proteins that are present in it but when the samples and serum was run with the drug shows only few bands, not all 6, were observed and not all samples have given bands. Thus indicating there could have been interaction of the drug with some of the proteins such as Alpha 1, Alpha, Beta protein and Serum Albumin.

Human serum albumin plays a major role as a reservoir. They help in the transport of endogenous substances like bilirubin, fatty acids and exogenous substances such as drugs, nutrients in the blood. Thus the binding of the drug to the serum albumin determines the pharmacodynamics and pharmacokinetic properties of the drug.



(A) (B) (C)

Figure 4: A- Showing the standard peaks observed for the different types of proteins that constitute the serum and the bands that was obtained after performing Native PAGE. B- The above figure shows Serum as control and serum with Sevelemer carbonate and samples as follows, *Citrulluslanatus*seeds, *Helianthusannus*seeds *Linumustatissimum*seeds, *Momordicacharantia,Musaparadisiaca* stem and *Schediumedule.* C- The above figure shows Serum as control and serum with SevelemerHCl and samples as follows, *Citrulluslanatus*seeds, *Helianthus annus*seeds *Linumustatissimum*seeds, *Momordicacharantia,Musaparadisiaca* stem and *Schediumedule.*

Drug-albumin interaction appears to occur at one or more specific binding sites. Nature of the drug binding sites is specific in their size, charge, location, hydrophobicity or changes that occur during the binding of a drug. These changes help in the analysis of drug-drug interaction or drug-protein binding in diseased states.

SUMMARY:

In the present investigation several parameters were taken to study the drug, its effect in combination with the selected samples, interaction with serum proteins and swelling properties. The solubility of the drugs – Sevelemer carbonate and Sevelemer HCl was performed using several solvents and buffers and the compounds were found to be insoluble to all of them. This may be due to their complex structure made from many monomers having no free sites to bind to. To confirm the interaction of protiens and the drug Native PAGE was performed and was observed that the standard serum shows 6 bands corresponding to the different proteins present in it such as Albumin, Alpha 1, Alpha 2, Beta and Globulin protein. The interaction of the drug with serum as well as with samples shows the presence of only few bands and not all 6, this may be due to the interaction of that protein with the drug. The swelling of the drug was also observed under Phase contrast microscope, where the drug when visualised under its normal state was seen as a mass of particles and once water was added it showed disintegration patterns and the drug dissociates

rapidly. in 15 minutes there was swelling of the minute particles of the drug seen which was coated by a layer of the solvent.

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