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Evaluation Of Effect Of Nanoparticulate Clay On Cholesterol Binding Capacity Of Colesevelam HCl.

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ABSTRACT

Colesevelam HCL is non-absorbable lipid lowering substance that binds to bile acids in the intestine, impending their reabsorption. It is commonly used bile acid sequestrant in high doses. Cholesterol is the sole precursor of bile acids. Epidemiological investigations have established that cardiovascular morbidity and mortality vary directly with the levels of total-C and LDL-C, and inversely with the level of HDL-C. In the present study, it is attempted to evaluate the combined effect of nanoparticulate clay (nanoclay) with Colesevelam HCL in hypercholesterolemia. The study includes the evaluation possible synergistic action of drugs binding properties with lipids in aqueous conditions. It was observed that the presence of nanoclay has shown very negligible variation on Cholesterol binding capacity of drug at different pH (1.5 to 7.5) of the medium that covers the entire GI Range. The work suggests the possible alternative to the present combination of Colesevelam HCL and statins in hypercholesterolemia.

Keywords: Non systemic drug, Colesevelam HCL, Cholesterol binding capacity, Nanoparticulate clay, Hypercholesterolemia.

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INTRODUCTION

Non-absorbable drugs are delivered most of the times by oral administration. They are often administered in combination to exhort their therapeutic effects locally in the GI tract. In contrast to traditional drugs, which are designed to be rapidly absorbed, to achieve therapeutic plasma levels, and then be limited by multiple pathways, non-absorbable drugs are designed to minimize systemic exposure. The non - absorbable drugs are used extensively to treat systemic metabolic and mineral disorders as well as in the diseases of the GI tract. Early examples of non-absorbable drugs were relatively unsophisticated cation and anion exchange resins that remove bile acids or potassium ions from the lumen of the gut for the treatment of hypercholestemia and hyperkalemia, respectively. The best example of bile acid sequestrant is Colesevelam HCL. Colesevelam HCL is categorized class IV drug according to BCS classification system which indicate that it possess low solubility and low permeability. It is non-systemic drug which acts on GI tract.The mechanism of Colesevelam HCL for its bile acid sequestrant action is its interaction with LDL like Cholesterol and other lipids in intestine. There is scope to evaluate its binding with LDL like Cholesterol at various pH conditions and it will be interesting to evaluate the effect of nanoparticulate clay or any other nanomaterial on binding properties and extent of Colesevelam HCL.

There are chances that the presence of Nanoclay may prove to be enhancing the extent of binding of Colesevelam HCL with the lipids. In the study Cholesterol is used as a model lipid representing LDL. In the present study, this is attempted and the evaluation parameters are compared by keeping in mind the synergestic action of substance adsorption properties with lipids in aqueous conditions. In the proposed work nanoparticulate form is planned to be used for improvisation of binding of Colesevelam HCL with intestinal lipids.

MATERIALS AND METHODS

MATERIALS

Colesevelam HCL was obtained from Emcure Pharmaceutical Pune, India as a gift sample. Cholesterol and all other chemicals and solvents used in the present study were of analytical grade and procured form Research Lab Fine Chem Mumbai.

EXPERIMENTAL

Preparation of physical mixture of Colesevelam HCL-cholesterol mixture for 1:1and1:4 ratios for room temperature:

Method of preparation:

For development of Colesevelam HCL-Cholesterol physical mixture various drug: Lipid ratio were used and the amount of drug and lipid was kept constant.

In the first step, the physical mixture was prepared by incorporating Colesevelam HCL(100mg) into Cholesterol(100mg) having ratio 1:1 and Colesevelam HCL(100mg) into Cholesterol(400mg) having ratio 1:4 respectively. Keep activation for 24 hrs at 30^{o}C in orbital shaker.

Procedure:

After 24hrs (Equilibrium time) 10 mg of the mixture was dissolved in 10 ml ethanol with continuous stirring on magnetic stirrer and also sonicated to ensure maximum solubilisation of Cholesterol. This dispersion was then filtered to collect the residue. Residue was washed with distilled water and dried at room temperature. By using this residue standard concentrations100-300ug/ml of cholesterol were prepared. This experiment was carried out at temperatures like at room temperature (30^{o}C) The cholesterol content was estimated by using UV analysis method at 242nm.

Variables:

Effect of temperature: The physical mixture of Coleseveam HCL: Cholesterol physical mixture various drug; Lipid ratio were used and the amount of drug and lipid was kept constant.

In the first step, the physical mixture was prepared by incorporating Colesevelam HCL(100mg) into Cholesterol(100mg) having ratio 1:1 and Colesevelam HCL(100mg) into Cholesterol(400mg) having ratio 1:4 respectively. Keep activation for 24 hrs at 30°C in orbital shaker.

Procedure:

After 24hrs (Equilibrium time) from that mixture 10 mg of the mixture is dissolved in 10 ml ethanol with continuous stirring on magnetic stirrer and then sonicate on probe sonicator. This dispersion was then filtered. ,residue was washed with distilled water and dried at room temperature. And then prepared a stock solutions100ug/ml, 200ug/ml, 300ug/ml of Colesevelam HCL:Cholesterol physical mixture for 1:1 and 1:4 ratios at 242nm.With reference to above method. Further study is carried out at temperature30°C for each drug:lipid for 1:1 and 1:4 ratios at 242nm. As shown in table:

B) Preparation of physical mixture of Colesevelam HCL-cholesterol mixture for 1:1and 1:4 ratio for different temperature:

Method of preparation:

For development of Colesevelam HCL-Cholesterol physical mixture various drug;Lipid ratio were used and the amount of drug and lipid was kept constant.

In the first step, the physical mixture was prepared by incorporating Colesevelam HCL(100mg) into Cholesterol(100mg) having ratio 1:1 and Colesevelam HCL(100mg) into Cholesterol(400mg) having ratio 1:4 respectively. Keep activation for 24 hrs at R.T. (30°C),R.T.+10(40°C),R.T.+20°C,(50°C)

Evaluation of Cholesterol binding capacity.

Effect of temperature: The physical mixture of Coleseveam HCL: Cholesterol physical mixture various drug:Lipid ratio were used and the amount of drug and lipid was kept constant.

Procedure:

After 24hrs (Equilibrium time) 10 mg of the mixture was dissolved in 10 ml ethanol with continuous stirring on magnetic stirrer and also sonicated to ensure maximum solubilisation of Cholesterol. This dispersion was then filtered to collect the residue. Residue was washed with distilled water and dried at room temperature. By using this residue standard concentrations of cholesterol were prepared. This experiment was carried out at various temperatures like at room temperature (30°C) and at 40°C, 50°C also. The cholesterol content was estimated by using UV analysis method at 242nm.

Effect of pH: The physical mixture of Coleseveam HCL: Cholesterol physical mixture various drug;Lipid ratio were used and the amount of drug and lipid was kept constant.

Procedure:

Three different flasks A, B, C were used to prepare dispersion containing 500mg mixture with in 50ml buffer having pH 1.5, 5.8, 7.4 respectively. These flasks were kept for continuous stirring on magnetic stirrer and also sonicated to attain the equilibrium. This dispersion was then filtered and residue was collected and then washed with distilled water and dried at room temperature. The cholesterol content was estimated by using UV analysis method at 242nm.

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C) Evaluation of cholesterol binding capacity of Colesevelam HCL in presence of Nanocarrier.

Method of preparation:

For development of Colesevelam HCL-MMT Clay physical mixture for drug:nanocrrier ratio were used and the amount of drug and nanocarrier was kept constant.
In the first step, the physical mixture was prepared by incorporating Colesevelam HCL(100mg) into MMT Clay(100mg) having ratio 1:1. Keep activation for 24 hrs at R.T. (30°C),R.T.+10(40°C),R.T.+20°C,(50°C).

Comparison of Cholesterol binding capacity of drug in presence and in absence of nanocarrier.

The Cholesterol binding capacity of drug is compared with the in presence of nanocarrier and in absence of nanocarrier.

RESULT AND DISCUSSION

Preparation of physical mixture of Colesevelam HCL: Cholesterol by using (1:1) ratio.

The Cholesterol was found to be 40% for 100ug/ml solution which is prepared from the stock solution prepared by using residue. The Cholesterol content was found to be 36% for 200ug/ml solution which is prepared from the stock solution prepared by using residue.

The cholesterol content was found to be 32% for 300ug/ml solution which is prepared from the stock solution prepared by using residue. The above results showed that there is less variation in the cholesterol content for different concentrations (100- 300ug/ml) This shows that the binding is saturable that is capacity limited and not linear with the concentration.

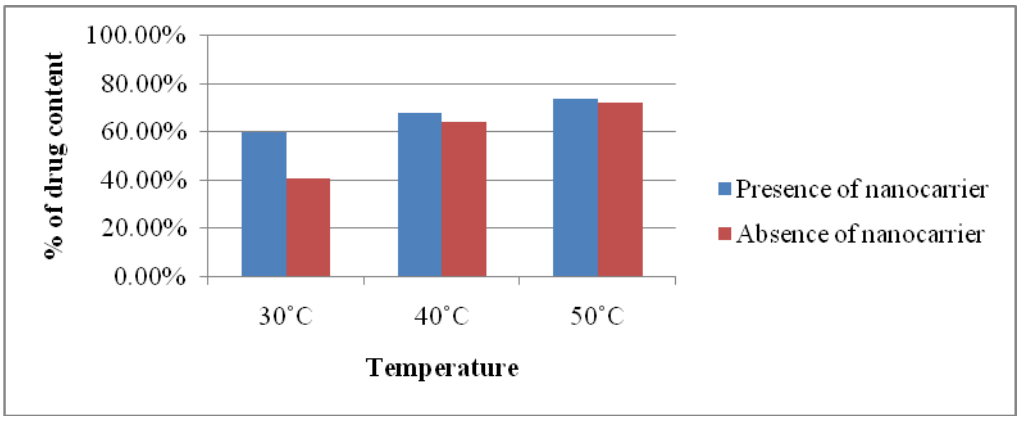

Figure 1: Comparison of Cholesterol binding capacity of drug in presence and absence of nanocarrier at various temperature.

Preparation of physical mixture of Colesevelam HCL : Cholesterol by using (1:4) ratio.

The Cholesterol was found to be 47% for 100ug/ml solution which is prepared from the stock solution prepared by using residue. The Cholesterol content was found to be 73% for 200ug/ml solution which is prepared from the stock solution prepared by using residue.

The cholesterol content was found to be 86% for 300ug/ml solution which is prepared from the stock solution prepared by using residue. The above results showed that there is variation in the cholesterol content for different concentrations (100- 300 ug/ml) This shows that the binding is attained an equilibrium and the excess of cholesterol is showing the increased content (percentage) with respect to increase in the concentration (100-300 ug/ml.)

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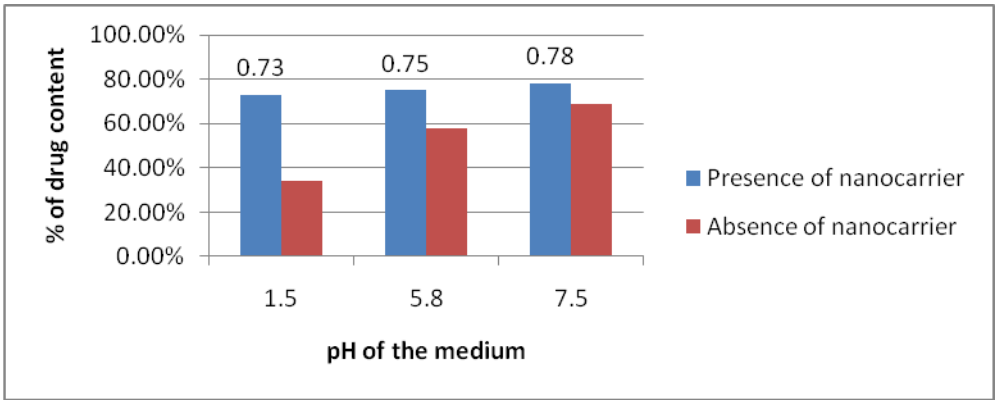

Figure 2: Comparison of Cholesterol binding capacity of drug in presence and absence of nanocarrier at various pH.

From results of cholesterol content determination after using various ratios of drug and chol esterol shows that the higher percentage of cholesterol observed for 1:4 ratio is only due to excess amount of cholesterol used in the physical mixture. This was confirmed by performing blank experiment in which drug was not used. Therefore, for the further analysis 1:1 of drug and Cholesterol was used.

Table 1: Percentage of Cholesterol content obtained at 1:1 ratio

Concentrations	Percentage of Cholesterol content Ratio (1:1)*
100ug/ml	40%
200ug/ml	36%
300ug/ml	32%

Table 2: Percentage of Cholesterol content obtained at 1:4 ratio

Concentrations	Percentage of Cholesterol content Ratio (1:4)
100ug/ml	47%
200ug/ml	73%
300ug/ml	86%

Preparation of physical mixture of Colesevelam HCL-Cholesterol mixture for 1:1ratio at different temperature

The percentage of Cholesterol content in batch A1, A2, A3, was found 41%, 64%, 72% respectively. Direct relationship was observed between binding capacities of drug with cholesterol with respect to temperature. Therefore, one of the variables for the evaluation of binding of drug with cholesterol can be temperature. The maximum cholesterol content was observed for batch A3 (50^{o}C) that is 72%

Tabel 3: Evaluation of cholesterol binding capacity of Colesevelam HCL -Cholesterol Mixture with different temperature

Batch code	Drug (mg)	Lipid (mg)	Drug:Cholesterol ratio	Temperature	Percentage of drug content
A1	100	100	1:1	30^{o}C	41%
A2	100	100	1:1	40^{o}C	64%
A3	100	100	1:1	50^{o}C	72%

Preparation of physical mixture of Colesevelam HCL-cholesterol mixture for 1:1ratio at different pH

The percentage of cholesterol content in batch B1, B2, B3, were 34%, 58%, 69% respectively. Direct relationship was observed between binding capacity of cholesterol with drug and pH of the medium. Therefore, one of the variables for the evaluation of binding of drug with cholesterol can be pH of the medium. The maximum cholesterol content was observed for batch B3 (pH 7.5) that is 69 %

Table 4: Evaluation of cholesterol binding capacity of Colesevelam HCL -Cholesterol Mixture with different pH

Batch code	Drug (mg)	Cholesterol (mg)	Drug:Cholesterol ratio	pH	Percentage of drug content
B1	500	500	1:1	1.5	34%
B2	500	500	1:1	5.8	58%
B3	500	500	1:1	7.5	69%

Evaluation of Cholesterol binding capacity of Colesevelam HCL in presence of Nanocarrier with different temperatures

The percentage of Cholesterol content in batch C1, C2, C3, was found 60%, 68%, 70% respectively. Direct relationship was observed between binding capacities of drug with cholesterol with respect to temperature. Therefore, one of the variables for the evaluation of binding of drug with cholesterol in presence of nanocarrier can be temperature. The maximum cholesterol content was observed for batch C3 (50^{o}C) that is 74%

Table5: Evaluation of Cholesterol binding capacity of Colesevelam HCL in presence of Nanocarrier with different temperature

Batch code	Drug (mg)	Nanocarrier (mg)	Drug: Nanocarrier ratio	Temperature	Percentage of drug content
C1	100	100	1:1	30^{o}C	60%
C2	100	100	1:1	40^{o}C	68%
C3	100	100	1:1	50^{o}C	74%

Evaluation of cholesterol binding capacity of Colesevelam HCL:MMT Clay Mixture with different pH

The percentage of cholesterol content in batch D1, D2, D3, were 73%, 75%, 78% respectively. The above results showed that there is less variation in the cholesterol content for standard concentrations (100ug/ml) by using various pH of the medium like pH 1.5, 5.8, 7.4. This shows that the binding is not dependent on the pH of the medium and not linear with the change in pH of the medium. Therefore, pH of the medium was not suitable variables for the evaluation of binding of drug with cholesterol.

Table 6: Evaluation of cholesterol binding capacity of Colesevelam HCL:MMT Clay Mixture with different pH

Batch code	Drug (mg)	Nanocarrier (mg)	Drug:Nanocarrier ratio	pH	Percentage of drug content
D1	500	500	1:1	1.5	73%
D2	500	500	1:1	5.8	75%
D3	500	500	1:1	7.5	78%

Compasrison of Cholesterol binding capacity of drug in presence and in absence of nanocarrier (at various temperatures)

From the results it can be understood that the presence of nanocarrier has shown significant effect on binding capacity of cholesterol with drug. This effect of presence of nanocarrier was observed to be increasing with increase in the temperature i.e, at 50^{o}C. The cholesterol binding capacity was found to be maximum in comparison with room temperature (30^{o}C).At room temperature it was observed that the presence of

nanocarrier has shown substantial improvement in cholesterol binding capacity in comparison with higher temperature like at 40°C,50°C).

Comparison of Cholesterol binding capacity of drug in presence and in absence of nanocarrier (at various pH of the medium)

From the results it can be understood that the presence of nanoclay has shown significant effect on binding capacity of cholesterol with drug. This effect of absence of nanoclay was observed to be increasing with increase in the pH i.e.at 7.5.. The Cholesterol binding capacity was found to be maximum in comparison with pH 1.5.

It was observed that the presence of nanoclay has shown very negligible variation on Cholesterol binding capacity at different pH of the medium (1.5-7.5).This shows that use of inert nanocarrier like nanoclay may improve the cholesterol binding capacity of Colesevelam HCL.

CONCLUSION

Colesevelam HCL is a nonsystemic drug which is commonly used as bile acid sequestrants. It binds to bile acids in the intestine and helps in inactivation of them and helps in their excretion in to faeces.In the present study, the combined effect of nanocarrier (nanoclay) with Colesevelam HCL in hypercholesterolemia is evaluated. The study includes the evaluation possible synergistic action of drugs binding properties with lipids in aqueous conditions. From the results it can be understood that the presence of nanoclay has shown significant effect on binding capacity of cholesterol with drug. It was observed that the presence of nanoclay has shown very negligible variation on Cholesterol binding capacity of drug at different pH (1.5 to 7.5) of the medium that covers the entire GI Range.

The study encourages the use of inert nanocarrier like nanoclay for enhancing cholesterol binding capacity of Colesevelam HCL throughout the GI tract. This study may be an initiative for providing a better alternative to the present combination of Colesevelam HCL with statins.

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