Bioavailability enhancement of rosuvastatin by complexation with cow ghee

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Abstract
The objective of the study was to increase the bioavailability of selected drug rosuvastatin by preparing complex of drug with ghee. Dissolution study was done by using paddle apparatus USP type 2 and permeation study was performed by everted intestinal method. The different ratios of complexes were prepared by solvent evaporation method and evaluated. The complexes were Rosuvastatin calcium with native ghee complex(1:1 to 1:5), rosuvastatin calcium with oxidized ghee complex (1:1 & 1:5), and complex with adsorbate rosuvastatin calcium with MCC and oxidized Ghee complex (1:1:1) and rosuvastatin calcium with aluminum magnesium silicate and oxidized ghee complex (1:1:1). In dissolution study of rosuvastatin calcium the percentage drug release was in the range from 35% at 15 minutes and 98% within 90 minutes; while in case of rosuvastatin calcium with native cow ghee and oxidized cow ghee, the % drug releases decreased to93% to 52% at 90 minutesand 93% to 55% at 90 minutes respectively. Thus the prepared complexes sustained the dug release. The permeation study showed that in the presence of ghee the permeation of rosuvastatin showed 96% release at 105 minutes and 93% at 75 minutes. Results of dissolution study proved that complexation with oxidized ghee led to maximum increase in bioavailability as compared to native ghee.

Keywords: Permeation, Dissolution, Ghee, Bioavailability, Rosuvastatin, Complexation.

Introduction
The rate of absorption and bioavailability of poor water soluble drug is that the major drawback encountered with formulation development of recent chemical entities.[1] Solubility and permeability is one among the necessary parameters to realizedesired concentration of drug in circulation for achieving needed medicine response. Poor water soluble drugs need high doses so as to achieve therapeutic plasma concentrations once oral administration.

Rosuvastatin is 3-hydroxy-3methylglutaryl coenzyme A- reductase inhibitor, developed for the treatment of dyslipidemia which reduces LDL-C and triglycerides (TG) and at raising HDL-C levels thereby. it's a selective and a competitive inhibitor of HMG-CoA reductase have poor water soluble drug that addressing drawback of low bioavailability (20%) as their dissolution is rate limiting issue. So, it becomes a demand to boost solubility of rosuvastatin.[3] Complexation with cow ghee was used to improve the poorly solubility of rosuvastatin. The literature survey and the previous work performed in laboratory indicate that the oxidation of ghee lowers the bad cholesterol present in the ghee therefore and decreases in chances of cardiovascular disease.[4] According to Ayurveda Ghee which is known as "Gritha", described as the best among lipid media due to its quality of inheriting and enhancing the drug potency.[7] It gives the more advantages to the body, its useas massage oil to cure many type disease like mantel disorder, hypertension etc. and ghee has capability to permeate endothelial cell present in the blood brain barrier. Based on the hypothesis that ghee can increase the permeability as well as bioavailability of the drugs; an attempt to investigate the dissolution study and permeation study of ghee and its complexes with rosuvastatin. Thus our aim and objective was to enhance the permeability of rosuvastatin by preparing the drug ghee complexes which in turn would enhance the bioavailability. For fulfil the aim and objective preparation of complexes and evaluation of physicochemical parameters of ghee and its complexes in which in-vivo drug release study and permeability were performed. The complex of the rosuvastatin was prepared in different ration with native ghee and oxidized ghee by solvent evaporation method.

Materials and Method
Material
Rosuvastatin calcium was procured from Mylan laboratory; Hyderabad. Cow ghee was purchased from Dharma Peth Nagpur. Ethanol, Acetone, Sodium chloride, Potassium chloride, Magnesium chloride calcium chloride sodium bicarbonate sodium hydrogen phosphate, Sodium di-hydrogen phosphate potassium di-hydrogen orthophosphate were purchased from Sudarshan chemical, Raipur (C.G).

Fresh slaughtered cockerel intestine bought from the nearest slaughter house which was kept in tyrode's solution at 20°C, till further use. Equipment and assembly for everted intestine was assembled using reservoir (made of glass and plastic container) having capacity of 250ml with controlling valve for monitoring the flow rate of solution go through the pipe to the assembly, the assembly was prepared in glass container in which inlet and outlet were made by clamping two pipe in both side of inverted intestine, one is connected to reservoir as inlet and another pipe are used as outlet. Electrical assembly are used for oxygen supply to the tissue through the aerator. A Shimadzu-1700 UV

visible spectrophotometer with 1 cm matched silica cells was used for spectrophotometric analysis.

Methods

Determination of λ Max and Preparation of Calibration Curve: An accurately weighed quantity of about 10 mg of rosuvastatin calcium was taken in 100 ml volumetric flask dissolved in sufficient quantity of ethanol then sonicated for 15 min and diluted to 100 ml with the same solvent so as to get the concentration of 100 µg/ml. This stock solution is used for making dilutions for calibration curve[17]. The standard solution of rosuvastatin calcium scanned at different concentration in the range of 200-400 nm and the λ max was determined.

Appropriate aliquots were pipette out from standard stock solution into the series of 10 ml volumetric flask and the volume was made up to the mark with ethanol to get concentration of 1-10 µg/ml of rosuvastatin calcium. Solutions of different concentrations for were analysed at their respective wavelengths and absorbance were recorded.[17]

Preparation of binary complexes at different ratio: Complexes of drug with native and oxidized were prepared. Fusion admixture of rosuvastatin calcium**:** ghee was prepared by melting the ghee in a beaker over a water bath maintained at 65-70°C temperature. To the molten ghee an equivalent amount of drug were added, and uniformly dispersed by continuous stirring to prepare binary mixture. The ration 1:1 to 1:5 w/w ratio was selected to maximize the likelihood of observing any interaction the fussed mixture was homogenized and allow to cool slowly to room temperature with stirring. The binary mixture was stored in amber colored glass bottles. Native ghee was oxidized by heating it in an electric stainless steel oven at 120°C for 50 hours. The complexes in same ratios were prepared by using oxidized ghee also.

***In-Vitro* Drug Release Study:** The paddle apparatus USP type 2 was adopted in this study. The release medium consisted of 900 ml of 0.1 N HCl solution. A known quantity from each batch of the drug were placed in chamber of the release apparatus and agitated at 60 rpm. At predetermined time intervals (15 min.), 5 ml of the release medium was withdrawn, appropriately diluted and absorbance determined at a respective wavelength using UV spectrophotometer. The volume of the release medium was kept constant by replacing it with 5 ml of fresh 0.1N HCl solution after each withdrawal. The release study was repeated using 0.1 N HCl solution as a release medium and the absorbance was determined at a known wavelength.

***Ex-Vivo* Intestinal Permeation Study**

Experiments using everted intestine method:[15] Six setup of pure drug sample and complexes drug: ghee were prepared and there quantity were taken according to their therapeutic dose respectively. The fresh cock intestine was bought from the slaughter house. A 10 cm of intestine was everted and the inlet and outlet were made by clamping two pipes in both side of everted intestine in which one is connected to reservoir as inlet and another pipe are used as outlet. This everted intestinal setup was dipped in beaker containing drug and 6.4 pH phosphate buffer. From the reservoir 7.4 pH phosphate buffer was supplied, about 15 ml volume was filled inside the everted intestine. After each 15 minutes interval the filled solution inside the intestine was taken out (about 5 ml), the flow rates were controlled by controlling valve having in reservoir pipe. Again makeup the volume with 7.4 pH phosphate buffer up to 15 ml inside the intestine.The procedure was followed for 135 minutes and intermittent samples were collected.After suitable dilutions by UV spectrophotometer the absorbance were taken at suitable wavelength.Concentration of drug in each sample was determined by regression equation.

Result and Discussion

UV Spectroscopy

The absorption maximum of drug in Ethanolic solution was found to be 325 nm.

***In-vitro* release study:** The release study of Control (pure drug) and their Complex with native ghee at different ratio (1:1, 1:2, 1:3, 1:4 and 1:5) were studied and calculated for drug release then graph were plotted between % Drug Release Vs Time.

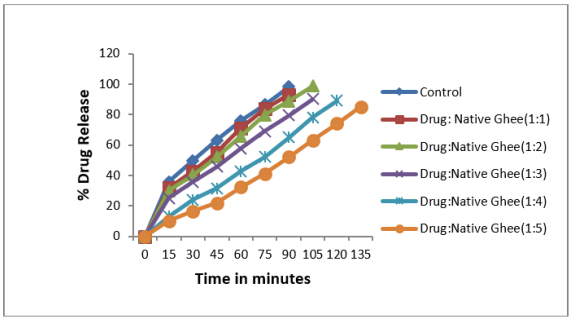

Fig.1: Drug release of rosuvastatin calcium complexes with native ghee

In the series of experiments it was observed that rosuvastatin calcium control drug shows 35.89% ,98.23% drug release at 15 minutes and 90 minutes respectively .When prepare a complex with ghee (1:1) it shows decreased % drug release as compare to control drug which was 31.87% at 15 minutes and 93.28% at 90 minutes. Again by increasing the ratio of ghee in complex 1:2 (Drug: Native Ghee) is shows 29.88% ,99.98% and 98.98% drug release at 15,90 and 105 minutes respectively . In drug ghee complex 1:3, it shows 25.14%, 79.56% and 90.22% in 15, 90 and 105 minutes which was lower than control drug release. In

1:4 ghee complexagain it decreases to 13.18%, 65.23%, 89.23% drug release in 15, 90, 120 minutes which means it takes more time to release the drug from complex. For 1:5 drug ghee complex it shows 10.12% Drug release at 15 minutes, 52.12 at 90 minutes and 85.21% at 135 minutes.

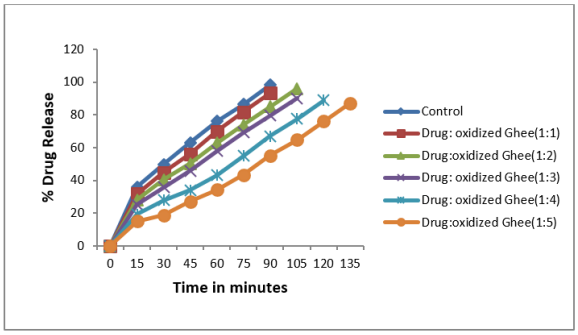

Fig. 2: % Drug release of rosuvastatin calcium complexes with oxidized ghee

The complexion of rosuvastatin calcium with oxidized ghee were studied and percent drug release were calculated. In this experiment drug : oxidized ghee(1:1) shows 31.87% drug release at 15 minutes and 93.28% at 90 minutes and the drug :ghee complex (1:2) shows 28.23% drug release at 15 minutes, 84.98% at 90 minutes and 96.23% at 105 minutes. Here percent drug release of drug: oxidized ghee 1:1 and 1:2 was lower than the control drug .and drug: oxidized ghee 1:1 is higher than the 1:2(drug: oxidized ghee) complex.again when increasing the ration of drug: ghee complex 1:3 it decreases % Drug release by 25.14%, 79.56 and 90.22% at 15, 90 and 105 minutes. In 1:4 ghee complex shows 19.18% drug release in 15 minutes and in 90 minutes it releases 67.15% drug than shows 88.98% at 120 minutes which means it takes more time to release the drug from complex. For 1:5 drug ghee complex it shows 15.12%. Drug release at 15 minutes and 55.13% at 90 minutes and then 76.23% at 120 min. than further releases drug 87.29 at 135 min.

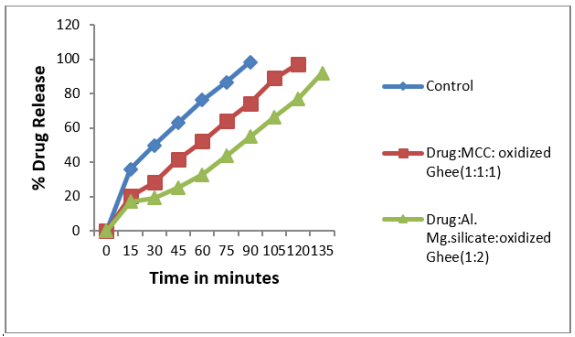

Fig. 3: Drug release of rosuvastatin calcium complexes with adsorbate and oxidized ghee

The complexion of rosuvastatin calcium oxidized ghee by adding absorbent were studied and percent drug release were calculated. In this experiment drug: Microcrystalline cellulose: oxidized ghee (1:1:1) shows higher percent Drug release as compare to control drug as 20.13% drug release at 15 minutes, 74.45% at 90 minutes and at 120 minutes it shows 97.23% of drug release . The drug: Aluminium Mg silicate: ghee complex (1:1:1) shows 16.98% drug release at 15 minutes and 54.87% at 90 minute and releases 91.88% of drug in 135 minutes which was lower than control drug. Here when compared that all rosuvastatin calcium complexes results, it was investigated that the percent drug release of drug: MCC: oxidized ghee 1:1:1 was lower than control drug but higher than complex of drug:Al.Mg.silicate:oxidized ghee(1:1:1) .

Permeation Study

The permeation of rosuvastatin calcium and there complexes at different ratio was studied and calculated for % drug permeation with respect of time. The Drug: Ghee complexes 1:1, 1:2, 1:3, 1:4, 1:5 were prepared for the permeation study.

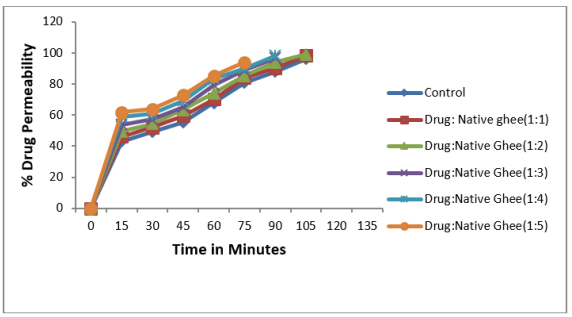

Fig. 4: Permeation of rosuvastatin calcium: native ghee complexes at different ration

In the series of experiments regarding permeability of rosuvastatin calcium it was observed that rosuvastatin calcium controldrug shows permeability as 43.21% in 15 minutes, 90.44 at 90 minutes. It completely releases the drug within in 105minutes as 98.23%. In complex ratio 1:1 it shows 46.23% drug permeation at 15 minutes, 90.44% at 90 minutes and 98.23% permeation at 105 minutes. In 1:2 (drug :ghee complex) it shows 49.58% drug permeation at 15 minutes,93.88% at 90 minutes and completely release drug with in120 minutes. In drug: ghee (1:3)% drug permeation at 15 minutes it was 53.89%.96.88% at 90 minutes. In complex 1:4(drug:ghee) at 15 minutes the % drug release was 58.94 and increasingly it goes to 98.32% at 90 minutes of their % drug release . In drug: ghee (1:5)% drug release at 15 minutes it was 61.89%, 93.88% at 90 minutes. From all the permeation study we can conclude that the permeation of drug increases ad increase in the amount of ghee in drug: ghee complex ration. Rosuvastatin calcium complex were also prepared with oxidized ghee. Drug:ghee(1:1) and drug :ghee (1:2) and % drug permeation were observed.

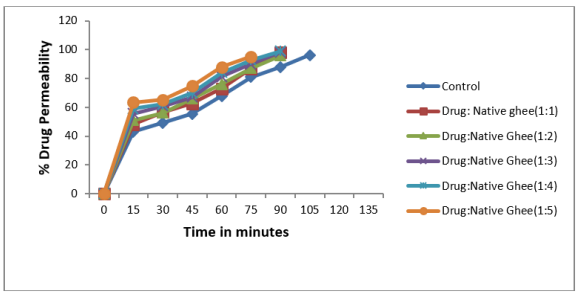

Fig. 5: Permeation of rosuvastatin calcium: oxidized ghee complexes at different ration

In this experiment the complexes of rosuvastatin calcium: oxidized ghee was analyzed for % drug permeation. In drug: oxidized ghee(1:1) the % drug permeation was 48.33% at 15 minutes,98.15% at 90 minutes . In drug:ghee (1:2) 51.14% drug permeates at 15 minutes 95.88% at 90 minutes . Here drug: oxidized ghee (1:1) was lower than the control drug. While increasing the ratio 1:3 drug:ghee complexes it increases their release by 55.78% at 15 minutes, 98.23 at 90 minutes. In 1:4 drug : ghee complex it shows 59.14% drug release at 15 minutes, 99.02% at 90 minutes .In 1:5 complex it again increases their release by 63.44% at 15 minutes ,95.33 % at 90 minutes which means it rapidly permeate the drug as the amount of ghee increases in complex ratio.

In permeation study of complexes the *adsorbate* were also used in this experiments there % drug permeate were observed.

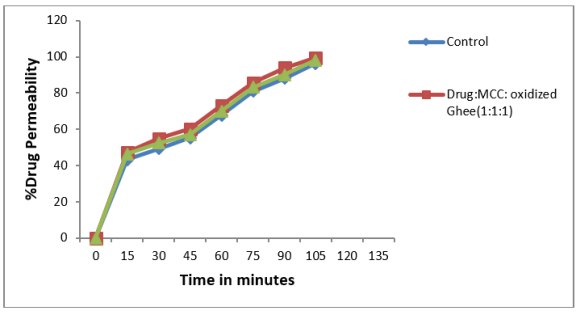

Fig. 6: Permeation of rosuvastatin calcium:oxidized ghee:mcc complexes

In this experiment the complexes 1:1:1 (rosuvastatincalcium: Microcrystalline cellulose: oxidized ghee) and 1:1:1 (rosuvastatin calcium:aluminum magnesium silicate:oxidized ghee) was analyzed for % drug permeation. In drug: Mcc:oxidized ghee(1:1:1) the % drug permeation was 47.25% at 15 minutes,93.88 at 90minutes which increases as 99.56% at 105 minutes. In (drug: Al. Mg. Silicate:oxidized ghee) 1:1:1.46.26% drug permeates at 15 minutes,90.14 at 90 minutes and 98.19% at 105 minutes. Here when compared that all rosuvastatin calcium complexes results, it was investigated that the percent drug permeation of drug: MCC:oxidized ghee 1:1:1 was higher than control drug but lower than drug: native ghee 1:1 complex.

Differential scanning calorimetry (DSC)

The native ghee exhibited endotherms at 11.28°C and 44.93°C followed by slanting line up to 300°C. In case of oxidized ghee, the peaks were sharp as compare to native ghee indicating probably purified material at 13.45°C and 45.04°C. The slanting line of native ghee was here almost straight up to 200°C and thereafter slightly decreasesup to 300°C probably indicating a product free of some residual material which was there in native ghee. The melting point of rosuvastatin calcium is 122°C and when complex with native ghee and oxidized ghee these peaks disappeared indicating a clear evidence that there is formation of drug ghee complex in both native and oxidized ghee.

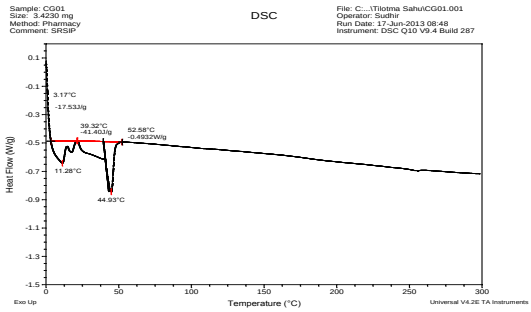

Fig. 7: DSC thermogram of native ghee

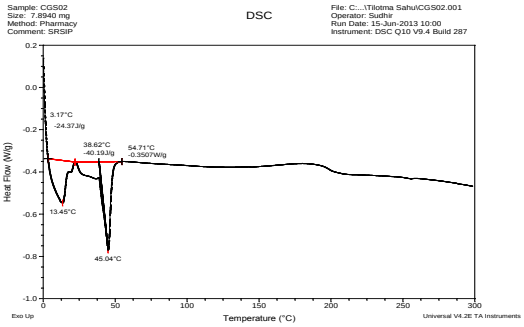

Fig. 8: DSC thermogram of oxidized ghee

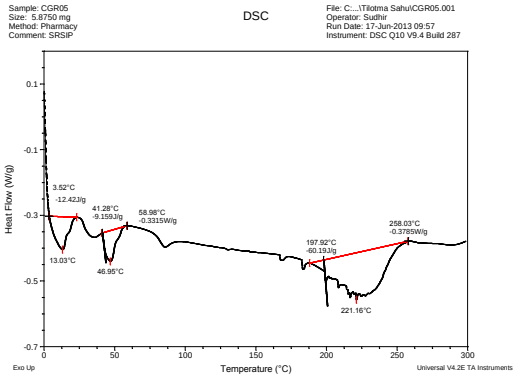

Fig. 9: DSC thermogram of rosuvastatain calcium:Native ghee complex (1:1)

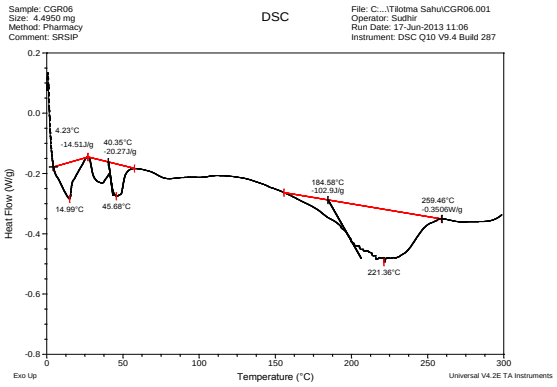

Fig. 10: DSC thermogram of rosuvastatain calcium:Oxidized ghee complex (1:1)

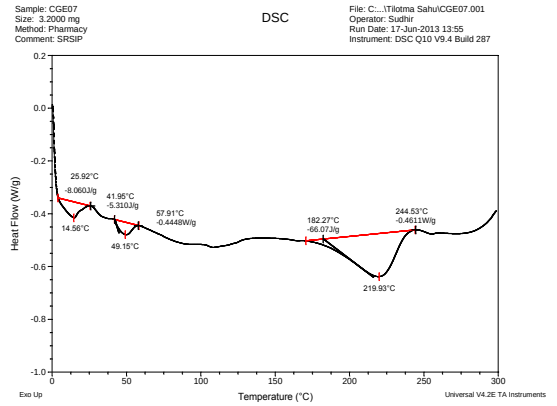

Fig. 11: DSC thermogram of Rosuvastatain Calcium:MCC:Oxidized ghee complex (1:1:1)

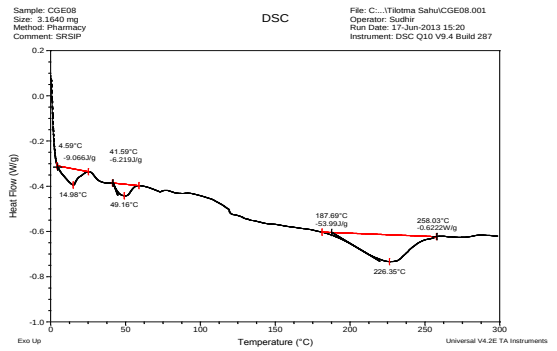

Fig. 12: DSC thermogram of RosuvastatainCalcium:Aluminum magnesium silicate:Oxidized ghee complex (1:1:1)

Discussion and Conclusion

Ghee contain sufficient amount of saturated fats and cholesterol and enhance risk factor for cardiovascular disease.[8] Therefore oxidizing ghee by heating it in an electric stainless steel oven at 120°C for 50 hours 17 times the concentration of oxysterol in the preparation. As per kumar administrating 2-5% oxidized ghee in diet decrease the total cholesterol level by 11-14% as compare to ground nut oil. Oxidized ghee contain enhanced free cholesterol ester fraction in mucosal cells, indicating that esterification process of cholesterol in the intestine is inhibited by ghee lipids(formed in oxidized ghee).[8] Therefore in present investigation, ghee has been oxidized and used further for research work with the view the oxidized ghee can used as pharmaceutical aid for preparation of formulations.

In ayurveda ghee is known to give soothing effect, hydrates the tissue and eases the drug permeation across the mucosal membrane as well as establishing the drug permeation for long period of time. In the work carried out in an attempt to prepare a stable drug ghee complex which may enhance drug permeability. The release of drug from Rosuvastatin calcium complex with ghee was shows lesser release of drug than control drug. When amount of ghee were incresease, the drug release was lowered, it may because of because of higher entrapment of the drug material. Complex made with oxidized ghee of rosuvastatin calcium exhibited nearly 82% drug release in 90 minutes in (1:1) drug: ghee complex, while 1:5 complex as obvious exhibited lower amount of drug release due to entrapment .Finally to take the sticky complexes to the formulation they were made to adsorb on the adsorbats so that the material becomes free flowing and compressible. The adsorbates choose were the microcrystalline cellulose and aluminium magnesium silicate. Dissolution study of the drug materials from these adsorbates indicates higher drug release by mcc (microcrystalline cellulose) as compare to aluminium magnesium silicate. Permeation study indicates just reverse of dissolution studyof rosuvastatin calcium. The amount of drug as compare to control permeated more as the amount of ghee increased. The amount of drug permeation through oxidized ghee is also higher in both cases. More interestingly the amount of drug release through adsorbates may be microcrystalline cellulose and aluminium magnesium silicate exhibited similar trend. The preliminary study of drug ghee complex of rosuvastatin calcium exhibited different physicochemical characteristics thereby interpreting the formation of complexes. Further the improved permeation supports their formulation steps and hence the drug ghee complexes can be used for improvement of drug therapeutics.

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