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UV-Vis Spectroscopic Investigation on Hydrolysis of Starch and Starch-Drug Interaction

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Gymnema sylvestre, *Pterocarpus marsupium* and *Madhumeha churnam* are the widely prescribed drugs for the treatment of diabetes mellitus by Siddha physicians. In order to estimate the drug activity of each compound, a novel concept namely hydrolysis of starch is applied. Chemically pure, wheat and corn starches are chosen along with human saliva as enzyme. Investigation of hydrolysis of these three kinds of starch by human saliva with and without the addition of antidiabetic drugs has been carried out with the help of UV-Vis technique. Keeping the starch concentration fixed, drug concentration was varied and the variation in absorbance of the starch solution, which is directly proportional to the rate of hydrolysis of starch, has been studied in the range 200-600 nm.

Key Words: Hydrolysis of starch, Human saliva-enzyme, UV-Vis spectral analysis, *Gymnema sylvestre*, *Pterocarpus marsupium*, *Madhumeha churnam*, Starch-drug interaction.

INTRODUCTION

As starch is composed of several glucose units, hydrolysis of starch can be brought about by both enzymes and acids. Enzymes which hydrolyse starch are amylases and these are present in saliva as ptyalin and in pancreatic juices as pancreatic amylase. Amylase acts on starch, hydrolyzing it into fragments of dextrin and ultimately to maltose molecules. They act at 1-4 glucosidic linkages. The products of hydrolysis are given below starting from starch:

Starch → Soluble starch → Amylodextrin + maltose + → Erythrodextrin + maltose ++ → Achrodextrin + maltose +++ → Maltose + maltose++++

The various products of hydrolysis of starch can be demonstrated by their colours obtained with iodine. Thus, the first product of soluble starch gives a blue colour. The next product amylodextrin gives a violet colour and third product erythrodextrin gives a red colour. Achrodextrin, which is the final product among dextrin, does not give any colour with iodine. It

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may be seen that at every stage starting from amylodextrin, maltose is also formed in increasing concentration as the hydrolysis progresses. The end product obtained by the hydrolysis of starch by enzyme is maltose. It may be noted that the hydrolysis of starch by acid goes a step further and the end product is glucose. For the hydrolysis of starch, certain anion like Cl^- and Br^- are required to act as activators. The optimum pH for amylase to hydrolyse starch in NaCl solution is 6. Complete hydrolysis of starch requires the presence of an intestinal enzyme called Oligo-1,6-glucosidase which acts at the 1-6 branching points and disintegrates starch completely[1].

Hydrolysis of starch mainly depends upon the reactive nature of an enzyme chosen. The activity of an enzyme, in turn, gets affected by a number of variable factors such as enzyme concentration, substrate concentration, covalent modification and pH value of enzymes, temperature and inhibitors of enzymes.

EXPERIMENTAL

The enzyme chosen is human saliva which is powerful in hydrolyzing carbohydrates. Initially the hydrolysis of starch starts in the mouth by saliva, which is first of the digestive secretions encountered by food. The chief organic constituents of saliva are the complex group of salivary proteins and the enzyme amylase, plyatin. Ptyalin is secreted in higher concentrations by the parotid glands. It belongs to a group of globulins having a molecular weight of about 50,000 and containing calcium ions in its structure which, when removed, inactivates the enzyme. This enzyme has an optimum pH of 6.8 and requires chloride ions for its activation. The α-amylase presents in saliva catalyses the breakdown of starch to maltose in a series of steps. Its concentration in saliva is independent of the rate of secretion even though prolonged periods of high carbohydrate intake may increase its concentration. Salivary amylase acts mainly on the polysaccharide starch, dextrin and to some extent on glycogen. It converts cooked starch into the disaccharide maltose but has no action on cellulose. The rapid ingestion of food leaves little time for digestion of starch to occur in the mouth. The digestion, however, continues in the stomach till the enzyme is inactivated by low pH and thus the digestive action occurs mainly in the stomach[2,3].

The starches selected are chemically pure starch, corn starch and wheat starch that are obtained from an established chemical firm. The enzyme saliva catalyses the hydrolysis of starch producing reducing sugar. The reducing sugar like maltose is coupled with 3,5-dinitro salicylic acid in alkaline medium. It produces an orange coloured complex. Its absorbance can be measured at 370 nm using UV-Vis spectrophotometer. The absorbance value is taken to be directly proportional to the extent of hydrolysis of starch or the activity of saliva[4].

The UV-Vis spectral measurements have been made over the region 200-600 nm for various concentrations of chemically pure starch, namely 1-5 % using Shimadzu UV 1601 spectrometer at Dr. Ceeal Analytical Laboratory, Chennai, India. The colour intensity due to hydrolysis of chemically pure starch is measured in terms of absorbance which is directly proportional to the rate of hydrolysis of starch by the enzyme. The same experimental procedure has been carried out with corn starch and wheat starch. The overlay UV-Vis spectra are presented in Figs. 1-3 and the results are tabulated in Table-1.

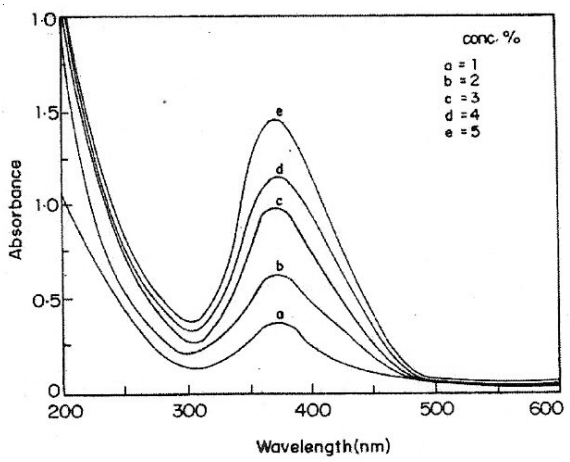

Fig. 1. UV-Vis characteristics of hydrolysis of chemically pure starch

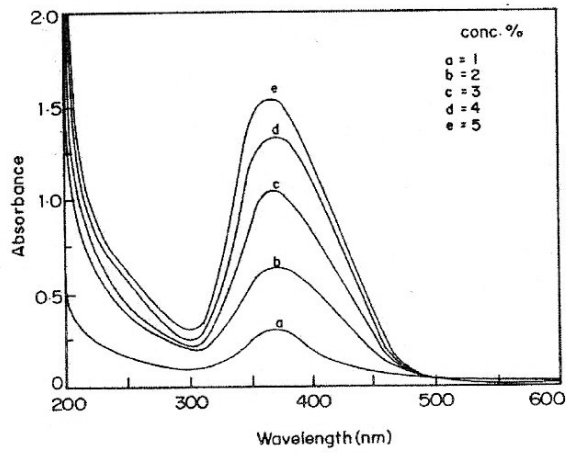

Fig. 2. UV-Vis characteristics of hydrolysis of corn starch

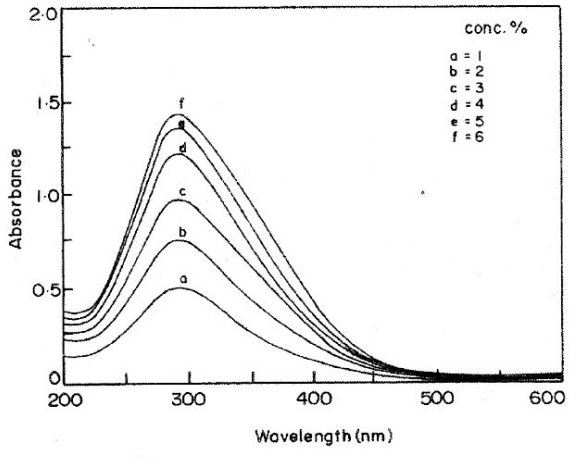

Fig. 3. UV-Vis characteristics of hydrolysis of wheat starch

TABLE-1
UV-VIS SPECTRAL MEASUREMENTS OF HYDROLYSIS OF STARCH BY SALIVA

Concentration of starch solution (%)	Quantum of saliva (mL)	Chemically pure starch		Corn starch		Wheat starch	
		λ_{max} (nm)	Absorbance	λ_{max} (nm)	Absorbance	λ_{max} (nm)	Absorbance
1	0.2	371.0	0.334	370.0	0.309	370.5	0.300
2	0.4	372.0	0.606	372.0	0.657	371.0	0.564
3	0.6	371.5	0.924	370.0	1.048	371.5	0.879
4	0.8	372.5	1.211	372.0	1.353	371.0	1.192
5	1.0	370.0	1.465	370.0	1.545	371.0	1.502

Interaction of drugs with hydrolysis of starch

Various varieties of starch chosen namely the chemically pure starch, corn starch and wheat starch were allowed to interact with some Siddha medicines namely *Gymnema sylvestre*, *Pterocarpus marsupium* and *Madhumeha churnam*. The activities of these drugs were estimated by the method of hydrolysis of starch chosen. The chemically pure starch solution was prepared at a concentration of 1 % and was hydrolyzed by 0.2 mL of human saliva. The UV-Vis spectral recording of this hydrolyzed starch was made in the region 200-600 nm. To this hydrolyzed starch solution, *Gymnema sylvestre* was added at 0.2 % concentration and its recording was done. Fixing the starch concentration at 1 %, drug concentration was varied from 0.2 to 1.8 % and spectral recording was made for

TABLE-2
UV-VIS SPECTRAL MEASUREMENTS OF CHEMICALLY PURE STARCH AND SALIVA WITH DRUGS
(STARCH CONCENTRATION FIXED)

Starch conc. (%)	Quantum of saliva (mL)	Before addition of drugs						After addition of drugs					
								Conc. of drug (%)	*Gymnema sylvestre*		*Pterocarpus marsupium*		*Madhumeha churnam*	
		λ_{max} (nm)	Absor-bance	λ_{max} (nm)	Absor-bance	λ_{max} (nm)	Absor-bance		λ_{max} (nm)	Absor-bance	λ_{max} (nm)	Absor-bance	λ_{max} (nm)	Absor-bance
1	0.2	370.5	0.243	371.0	0.200	370.0	0.275	0.2	372.0	0.213	370.5	0.178	370.5	0.220
								0.6	371.0	0.165	370.5	0.105	371.0	0.195
								1.0	370.0	0.101	–	–	370.0	0.115
2	0.4	371.5	0.521	370.0	0.469	370.5	0.680	0.2	372.0	0.433	370.5	0.358	371.0	0.502
								0.6	371.5	0.335	371.0	0.262	372.0	0.390
								1.0	371.5	0.224	368.0	0.204	371.5	0.278
								1.4	370.5	0.152	–	–	371.0	0.166
3	0.6	371.0	0.791	371.0	0.750	371.5	0.865	0.2	371.0	0.775	371.5	0.722	372.0	0.809
								0.6	371.0	0.684	370.5	0.623	371.5	0.697
								1.0	370.0	0.495	371.0	0.500	371.0	0.585
								1.4	370.5	0.351	370.0	0.451	372.0	0.473
								1.8	–	–	371.0	0.357	371.5	0.661
4	0.8	370.0	0.963	371.0	0.781	371.5	1.215	0.2	370.0	0.907	371.5	0.0722	372.5	1.116
								0.6	370.5	0.838	370.5	0.623	371.0	1.004
								1.0	371.0	0.759	371.0	0.500	372.0	0.892
								1.4	371.5	0.619	370.0	0.451	372.0	0.780
								1.8	372.0	0.502	371.0	0.357	370.0	0.668
5	1.0	371.5	1.280	372.0	1.115	370.0	1.450	0.2	371.5	1.204	372.5	1.001	370.5	1.402
								0.6	370.0	1.034	371.0	0.891	371.0	1.311
								0.8	370.0	0.897	372.0	0.781	372.0	1.199
								1.4	370.5	0.717	372.0	0.608	371.5	1.087
								1.8	370.5	0.659	372.0	0.479	371.0	0.975

TABLE-3
UV-VIS SPECTRAL MEASUREMENTS OF CORN STARCH AND SALIVA WITH DRUGS (STARCH CONCENTRATION FIXED)

Starch conc. (%)	Quantum of saliva (mL)	Before addition of drugs						After addition of drugs					
								Conc. of drug (%)	*Gymnema sylvestre*		*Pterocarpus marsupium*		*Madhumeha churnam*	
		λ_{max} (nm)	Absorbance	λ_{max} (nm)	Absorbance	λ_{max} (nm)	Absorbance		λ_{max} (nm)	Absorbance	λ_{max} (nm)	Absorbance	λ_{max} (nm)	Absorbance
1	0.2	371.5	0.270	372.0	0.389	371.5	0.309	0.2	368.0	0.207	370.0	0.350	372.0	0.283
								0.6	370.5	0.137	371.0	0.153	371.5	0.277
								1.0	371.0	0.109	370.0	0.040	–	–
								1.4	369.0	0.048	–	–	–	–
2	0.4	371.5	0.501	371.0	0.779	372.0	0.657	0.2	371.0	0.456	370.5	0.751	372.0	0.579
								0.6	370.5	0.343	371.0	0.545	371.0	0.449
								1.0	371.5	0.263	371.0	0.348	371.0	0.264
								1.4	370.5	0.154	371.0	0.151	372.0	0.184
								1.8	369.0	0.095	369.0	0.080	372.0	0.068
3	0.6	372.0	0.831	371.0	1.142	371.5	1.048	0.2	370.5	0.803	371.0	1.110	372.0	0.924
								0.6	371.0	0.606	370.5	0.903	371.0	0.721
								1.0	371.5	0.409	370.0	0.706	370.5	0.597
								1.4	370.5	0.212	371.0	0.509	370.0	0.353
								1.8	369.0	0.152	372.0	0.312	372.0	0.151
4	0.8	371.5	1.153	373.0	1.549	372.0	1.353	0.2	370.5	1.076	370.0	1.502	372.0	1.302
								0.6	371.0	0.879	371.5	1.305	371.5	1.151
								1.0	370.5	0.682	370.5	1.108	372.5	1.005
								1.4	372.0	0.485	369.0	0.911	370.5	0.727
								1.8	372.0	0.288	371.0	0.714	370.5	0.542
5	1.0	370.5	1.404	362.5	1.955	371.0	1.545	0.2	370.5	1.312	370.0	1.900	371.5	1.405
								0.6	371.0	1.115	370.5	1.703	372.0	1.224
								1.0	370.5	0.918	371.0	1.506	371.0	1.133
								1.4	370.5	0.721	370.0	1.309	372.0	0.805
								1.8	371.0	0.524	371.0	1.112	370.5	0.673

TABLE-4
UV-VIS SPECTRAL MEASUREMENTS OF WHEAT STARCH AND SALIVA WITH DRUGS (STARCH CONCENTRATION FIXED)

Starch conc. (%)	Quantum of saliva (mL)	Before addition of drugs						After addition of drugs					
								Conc. of drug (%)	*Gymnema sylvestre*		*Pterocarpus marsupium*		*Madhumeha churnam*	
		λ_{max} (nm)	Absor-bance	λ_{max} (nm)	Absor-bance	λ_{max} (nm)	Absor-bance		λ_{max} (nm)	Absor-bance	λ_{max} (nm)	Absor-bance	λ_{max} (nm)	Absor-bance
1	0.2	370.5	0.300	371.0	0.219	370.0	0.258	0.2	370.5	0.278	370.5	0.190	370.5	0.212
								0.6	370.5	0.138	369.0	0.063	371.5	0.122
								1.0	370.0	0.050	–	–	369.0	0.032
2	0.4	371.0	0.564	371.0	0.433	371.0	0.518	0.2	371.0	0.556	370.5	0.390	370.5	0.484
								0.6	371.0	0.436	371.0	0.263	371.0	0.394
								1.0	371.5	0.316	370.0	0.136	371.0	0.304
								1.4	371.0	0.196	–	–	371.0	0.214
								1.8	369.0	0.073	–	–	371.0	0.124
3	0.6	371.5	0.879	371.0	0.626	371.5	0.790	0.2	372.0	0.839	370.0	0.603	370.5	0.756
								0.6	371.5	0.719	371.0	0.476	370.5	0.666
								1.0	371.0	0.599	371.0	0.349	371.0	0.576
								1.4	371.0	0.479	371.0	0.222	371.0	0.486
								1.8	371.0	0.359	369.0	0.095	371.0	0.396
4	0.8	371.0	1.192	371.5	0.841	371.5	1.013	0.2	371.0	1.102	372.0	0.823	372.0	0.968
								0.6	370.5	0.962	371.0	0.696	371.5	0.878
								1.0	370.5	0.822	370.0	0.569	372.0	0.788
								1.4	371.0	0.682	371.0	0.442	370.0	0.698
								1.8	371.0	0.542	371.0	0.315	370.5	0.608
5	1.0	371.0	1.502	370.5	1.063	372.0	1.283	0.2	372.0	1.490	370.5	0.990	371.0	1.240
								0.6	372.0	1.316	371.0	0.863	371.0	1.150
								1.0	371.5	1.176	371.0	0.763	370.5	1.060
								1.4	371.5	1.036	371.5	0.609	372.0	0.970
								1.8	371.0	0.896	371.0	0.482	371.5	0.880

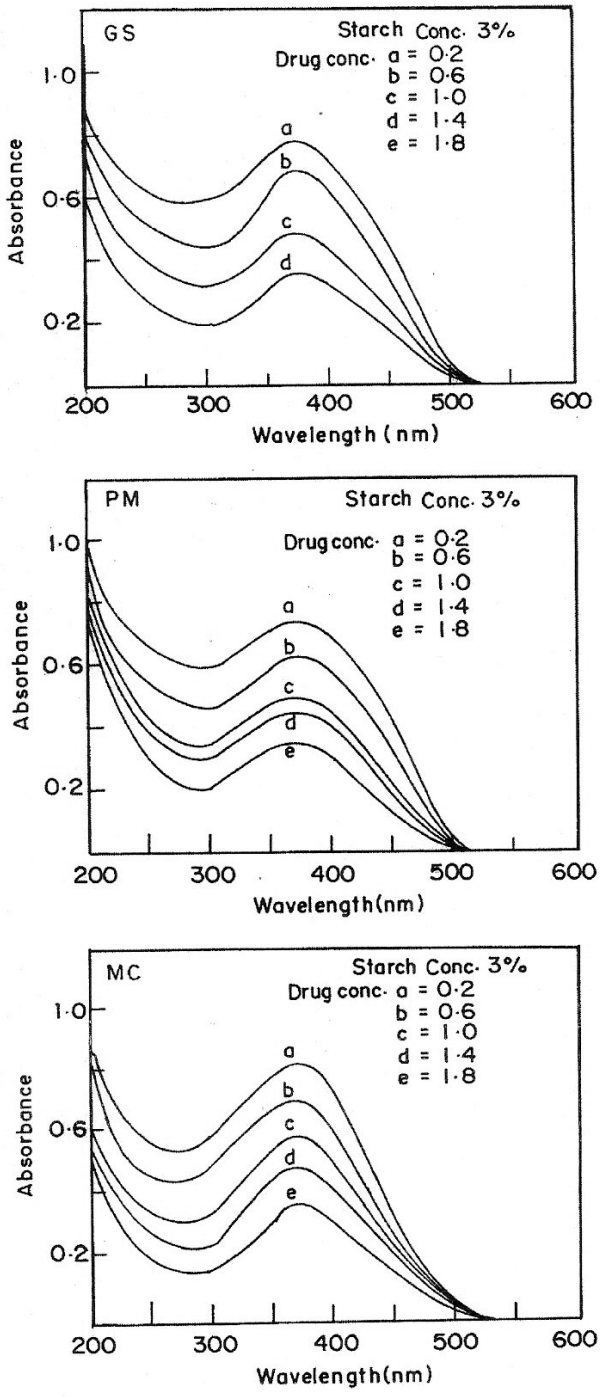

Fig. 4. UV-Vis drug interaction characteristics of chemically pure starch with *G. sylvestre* (GS), *P. marsupium* (PM) and *M. churnam* (MC)

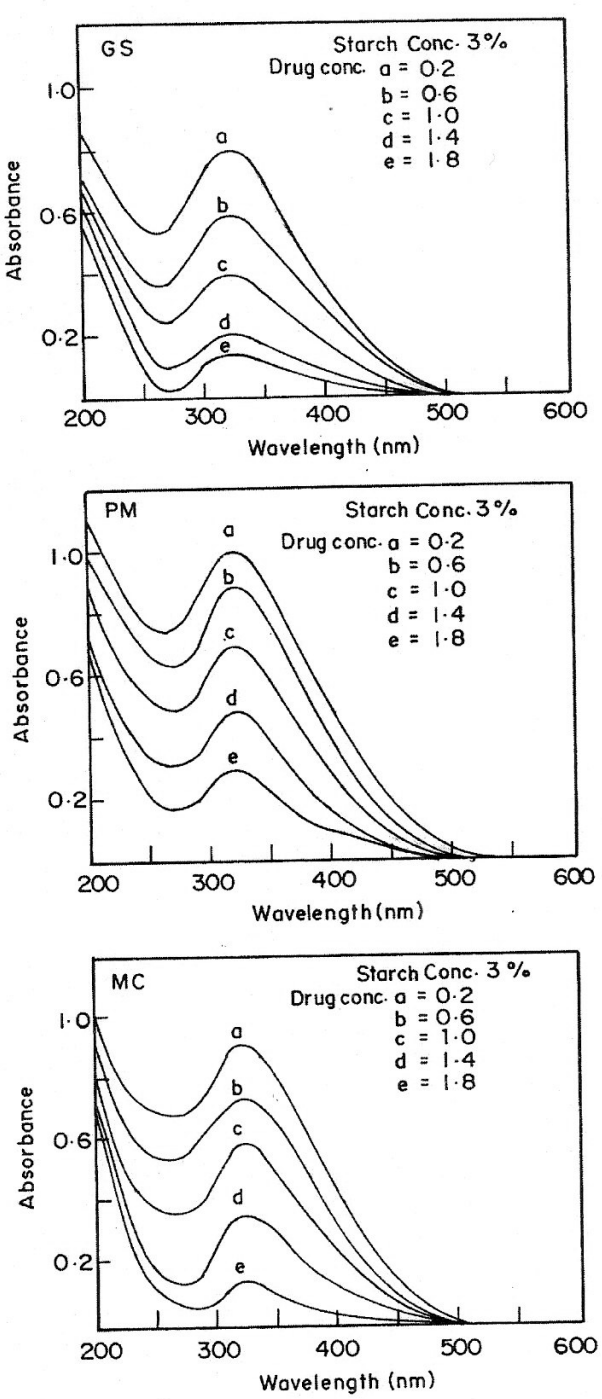

Fig. 5. UV-Vis drug interaction characteristics of corn starch with *G. sylvestre* (GS), *P. marsupium* (PM) and *M. churnam* (MC)

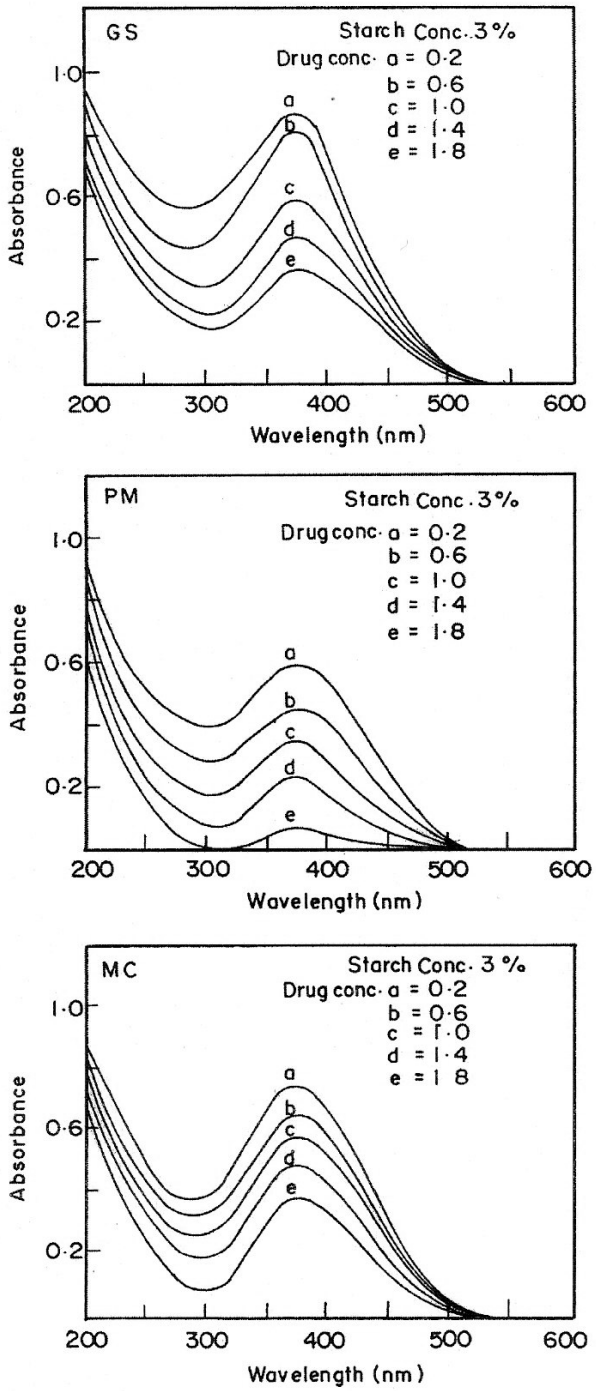

Fig. 6. UV-Vis drug interaction characteristics of wheat starch with *G. sylvestre* (GS), *P. marsupium* (PM) and *M. churnam* (MC)

each concentration of the drug. Concentration of starch was varied from 1 to 2, 3, 4 and 5 % and corresponding spectral recordings were made. The above procedure was carried out with *Pterocarpus marsupium* and *Madhumeha churnam*. The whole experimental procedure has been carried out for corn and wheat starch. The observations are tabulated in Tables 2-4 and the respective spectra are presented in Figs. 4-6.

RESULTS AND DISCUSSION

In the first phase of the experiment, the chemically pure, corn and wheat starch were hydrolyzed by the enzyme human saliva and UV-Vis spectral recordings have been made for various concentrations of starch as shown in Figs. 1-3. It is observed from Table-1 that, as the concentration of starch is increased, correspondingly, quantum of saliva is also to be increased in order to complete the hydrolysis activity. As the starch concentration increases, the absorbance of starch solution gets increased linearly, indicating the linear increase in the rate of hydrolysis or enzyme activity. This is due to the fact that as the substrate concentration increases, the rate of hydrolysis increases because greater number of substrate molecules collides with enzyme molecules so that high reaction will take place. At higher concentration, the enzyme molecules become saturated with substrate so that there are no free enzyme molecules. Further increase in substrate has no effect on the enzyme. Similarly as the enzyme concentration increases, the rate of hydrolysis increases, linearly because there are more enzyme molecules available to catalyze the reaction. At very high enzyme concentration, the substrate concentration becomes rate limiting so that the increase in rate gets stopped[5,6].

In the second phase, the starch-saliva solution was allowed to interact with drugs by keeping the starch concentration fixed at 1, 2, 3 %, *etc*. and the drug concentration was varied. It is observed from Tables 2-4 that the increase in concentration of drugs results in decrease in absorbance of starch and saliva solution whose concentration is kept fixed. Actually, the absorbance values of starch after addition of drugs are less than those values before addition of drugs. This implies that each drug is having its own impact on saliva activity or hydrolysis of starch. Even when the drug is present in small quantity, it is capable of reducing the rate of hydrolysis of starch and hence produces a decrease in absorbance as illustrated in Figs. 4-6. The reduction in the rate of hydrolysis of starch by the addition of any one of these three drugs indicates correspondingly the reduction of starch in to glucose units, which in turn will result in decrease of the amount of glucose being accumulated in blood. Thus lowering of blood sugar level from higher to normal value will occur in diabetic patients by the intake of these antidiabetic drugs.

Conclusion

From the UV-Vis spectral analysis of hydrolysis of starch, it is understood that the absorbance of starch can be reduced to a minimum with the increase in drug concentration and hence at one stage, for a particular concentration of drug, the absorbance would become zero. It implies that starch hydrolysis activity can be completely stopped by the intake of anyone of these three drugs at suitable dosage and hence prevents the reduction of starch into glucose units. This reduction in an indirect way prevents the accumulation of glucose units in the blood of a diabetic patient. Thus each drug makes an attempt to keep the blood sugar level at normal value and tries to prove itself as an antidiabetic medicine.

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