Original Research Article

Formulation and development of plasma volume expander using natural and modified starch from *Solanum tuberosum*

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Abstract

Background: To formulation and development of plasma volume expander (PVE) by using natural and modified starch from *Solanum tuberosum*. The function of blood circulation is to provide the needs of the body tissues and to maintain an appropriate environment in all tissue fluids of the body for the optimal survival and functions of the cells. Rapid restoration of the blood volume is necessary to decrease reduction in the amount of the blood. The PVEs are isotonic colloidal solutions, act by increasing the osmotic pressure of the intravascular compartment, which leads to the influx of the interstitial fluids through the capillary pore which, in turn, leads to the increase in the volume of the blood. Therefore, there is a need to discover the PVE with less side effects. The main aim of the present study is to use amylopectin as PVEs, fractionated from natural and modified starch obtained from *S. tuberosum.* **Methods:** The starch extracted from the normal grains and the tubers of potatoes was selected for the production of starch. Statistical analysis includes *in vitro* characterization that involves viscosity studies, plasma–product interaction, osmotic pressure detection, molecular weight–viscosity relationship, determination of weight average molecular weight, enzymatic interaction, and *in vivo* characterization such as toxicity studies and the effect of the products on the blood coagulation. The isolated starch and fractionated amylopectin were analyzed for the physicochemical characteristics. **Result and Conclusion:** The amylopectin fractionated from isolated starch from grains and tubers of potatoes can be used as PVE, as per the outcome of the study.

Key words: Amylopectin, fractionated starch, plasma volume expander, *Solanum tuberosum*

INTRODUCTION

The function of the blood circulation is to provide the needs of the body tissues. In certain pathological conditions such as acute bleeding, burns, sepsis, or any other kind of polytrauma, the reduction in the amount of the blood may result.[1-3] The rapid restoration of the blood volume is necessary in the above-mentioned cases. This restoration of the blood volume can be done by the whole blood as well as from the plasma substitutes.[4-7] The blood itself given is the best treatment in these cases where the restoration of the blood volume is necessary, as all the constituents such as blood coagulants, immunoglobulins, and electrolytes are provided by the blood in the optimum extent.[4] However, the major problem related to the whole blood transfusion is that the cross-matching of the blood is to be done prior to the blood transfusion.[8] In addition, the major risk of the transfusion of the blood is the transmission of the diseases such as hepatitis and HIV infection to the recipients.[7,9-12] Thus, in the case of the acute blood loss, the plasma substitute is preferred over the whole blood as the risk of transmission of these deadly diseases is avoided.[2,9] For the past two decades, the much of the research work is done in the field of the plasma substitute, and the various effects

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Access this article online

Quick Response Code:

Website: www.jpionline.org

DOI: 10.4103/2230-973X.195930

How to cite this article: Thombre NA, Vishwakarma AV, Jadhav TS, Kshirsagar SJ. Formulation and development of plasma volume expander using natural and modified starch from *Solanum tuberosum*. Int J Pharma Investig 2016;6:207-17.

of these plasma volume expanders (PVEs) on the physiology were studied.

In case of the emergency conditions such as shocks or accidents resulting in burns and hemorrhage cases, PVE has proved to be the lifesaving drug therapy. Hence, this lifesaving drug therapy should be free from the side effect, and thus, there is a need for the discovery of the new PVE.[6,13,14] The starch extracted from the normal grains and the tubers of potatoes was selected due to easy availability and economic method for the production of starch.[15] If these extracted products fulfill all the criteria which are necessary for any polymer to be used as the colloidal PVE solution, it will be a good approach in the field of the PVE research.

In this current research work, a new approach for the use of the amylopectin as PVE has been carried out. The glycogen and the amylopectin are the polymers which are similar in structure, only the branching of both the polymers is different. The amylopectin obtained from the fractionation of potato starch, wheat and maize starch, and oxidized and reduced starch, respectively, was used as PVEs. The starch rich in the amylopectin component is hydroxyl ethylated to form derivatives, which have the high degree of branching, and dextrans are used as PVEs. The outcome of the current study proved that it can persist in the body and can provide the necessary activity of compensating the loss of volume of the blood by acting as the PVE.

MATERIAL AND METHODS

Material

The plant of *Solanum tuberosum* was collected from the farmers of village Satpur, Nashik district, Maharashtra, India. The selected plants were authenticated from the Institute of Botanical Survey of India, Western Regional Centre, Koregaon Road, Pune - 411 001, Maharashtra, India. The chemicals used for the isolation, fractionation, and modification of starch were of analytical grade supplied by the Thomas Baker, Mumbai, India.

Isolation of starch

Starch was isolated from the potatoes (*S. tuberosum* L. Family *Solanaceae*) as described by Wallis *et al*. with slight modification.[16] The potatoes were washed thoroughly and the skins of the potatoes were removed. The pulps of the potatoes were crushed into the mixer into a fine slurry so that the starch stored in the potato cells gets released. The milky slurry obtained was then diluted with the freshly prepared distilled water to get the starch suspension at a concentration of 3–3.5% w/v. The slurry was passed through the sieve (mesh size #120). The obtained thick slurry was allowed to stand for 3 h. Further, the slurry was washed with the 0.01 M sodium hydroxide solution 0.01 M until the solution became clear for the removal of the endotoxins present, if any, into the solution, followed by washing of slurry with the distilled water for the removal of the alkali. The slurry was again centrifuged at a speed of 5000 rpm for 5 min. The white-colored damp mass was obtained which was dried in an oven at 40–45°C for 12 h. The brownish layer, if any, formed over the dried mass was scraped off and dried slowly at a temperature of 30°C for 54 h. The dried mass was then ground into fine powder and passed through the sieve (mesh size #120). The sieved powder was stored in a dry place (desiccators) till further use.[17,18]

Fractionation of the amylopectin from starch

Amylopectin was fractionated from the isolated starch from the tubers of potato. As starch is rich in amylopectin or amylose, the same was used for the preparation of PVE in the present research work.[19] The amylopectin was fractionated from the isolated starch by the procedure given in literature with a slight modification. Homogeneous suspension of the isolated starch at a concentration of 20% w/v was prepared. pH of the resulting solution was made alkaline with the addition of 0.5 M sodium hydroxide 0.5 M, followed by the addition of 10% v/v butanol. The mixture was heated at 60°C for 1 h with continuous stirring. After heating the suspension, clear solution was obtained, which was cool. Methanol was added to the above clear solution for the precipitation of amylopectin and then centrifuged at a speed of 5000 rpm for 10 min. The supernatant obtained by the centrifugation was swirled and amylopectin was re-precipitated by the addition of methanol. The precipitated amylopectin was then washed with the ethanol and with the 0.01 M sodium hydroxide solution subsequently. The pH of the solution was neutralized by the addition of 1% w/v hydrochloric acid. The precipitated amylopectin was air dried at 30°C in the hot air oven. Further, the dried amylopectin powder was passed through the sieve (mesh size #120) and kept in a dry place (desiccators) till further use. Butanol was added to the supernatant for the precipitation of the amylose. The obtained amylopectin from the fractionated starch was used for characterization and for the modification of starch by oxidation and reduction method.

Modification of starch by oxidation and reduction

Fractionated amylopectin was modified by the oxidation and reduction method.[20] One gram of isolated amylopectin was dissolved in 10 mL of distilled water. About 0.374 g of sodium periodate with 175 mm was slowly added to the above solution with continuous stirring for 3 h to form oxystarch. Further, the solution was diafiltered against water until the conductivity of the filtrate was 25 μs. The concentration of oxystarch solution was then adjusted at a concentration of 100 g/L. About 0.079 g of sodium borohydride was added to the 7.96 mL of oxystarch solution to obtain the desired concentration of sodium borohydride with 263 mm. The reaction mixture was stirred for 2 h. The resulting solution was diafiltered against water until the filtrate conductivity reached to 58 μs. The pH of the solution was then adjusted to 6.3 with hydrochloric acid, and starch solution concentration was adjusted to 3.39 mL/L. Finally, the chloride concentration was adjusted to 154 mM. The solution was then filtered aseptically into glass vials, stoppered, and stored at 4°C.

Physicochemical characterization of the potato starch

The potato starch and the modified starch were studied by using organoleptic test (color and odor test) and were identified by the iodine test and ultraviolet (UV) spectrophotometrically.[16,21]

Iodine test

The isolated starch, fractionated amylopectin, and modified starch were analyzed by iodine test for preliminary confirmation of starch. One gram of starch powder was suspended in 50 mL of distilled water and boiled for 1 min to form cloudy mucilaginous. About 0.05 mL of 0.01 M iodine solution was added to the 10 mL mucilage.[21]

Analysis by ultraviolet-visible spectrophotometer

The UV-visible spectrophotometer analysis was used to study the preliminary confirmation of isolated starch and its fractionated amylopectin. About 0.05 mL of 0.01 M iodine solution was added to the 10 mL mucilage and subjected to UV-visible spectrophotometric analysis in the visible range of 440–660 nm.[21]

Physicochemical evaluation

The physicochemical properties such as loss on drying, ash value, acid-insoluble ash value, oxidative substance detection, and the limit of iron content were determined according to the Indian Pharmacopeia, 2007.[21] The pH of 3% w/v fractionated amylopectin solutions and 3% w/v modified starch solutions was determined by Digital pH meter MK VI (Systronic, Ahmedabad, India). The total nitrogen content of amylopectin and modified starch was determined by the semi-micro Kjeldahl method. The total protein content was estimated by multiplying with the factor 5.6 according to the Indian Pharmacopeia, 2007.

Fourier transform-infrared spectra

The analysis of isolated starch, fractionated amylopectin, and modified starch solution was carried out by the infrared (IR) spectrophotometer for the determination of the functional groups present in the samples. IR spectra were recorded using FTIR 8300 (Shimadzu, Japan) spectrophotometer in the region of 4000 to 450 cm^{-1}. KBr pellets were obtained by blending and compressing a small amount of the above samples in KBr (1:10) on an IR press. The prepared pellets were placed in the pellet holder in the path of the light, and the spectra were recorded. The obtained spectra were compared with the standard spectrum reported in literature.

In vitro characterization

The *in vitro* characterization was performed on the fractionated amylopectin solution and modified starch solution.

Determination of the weight average molecular weight

The weight average molecular weight of the fractionated amylopectin solution and the modified starch solution was determined by using Mark–Houwink relationship, where the molecular weight–intrinsic viscosity relationship was studied.[22]

Intrinsic viscosity and molecular weight–viscosity relationship (Mark–Houwink relationship)

One percent of weight/volume fractionated amylopectin stock solution was prepared using 0.9% w/v sterile sodium chloride saline solution aseptically and filtered through the cellulose membrane filter having a pore size of 0.45 μm. The 1% w/v modified starch stock solution was prepared using distilled water and filtered through the cellulose membrane filter having a pore size of 0.45 μm. The obtained clear solutions were diluted to obtain 10 different concentrations, namely 0.01–0.1/g/dL. The Ostwald's viscometer was thoroughly cleaned with chromic acid solution and dried. The viscometer was mounted in vertical position on the stand. The viscometer was filled with water and sucked in the upper bulb up to the upper mark. The time taken in seconds for water to flow from the upper mark to the lower mark was counted. The same procedure was repeated for the fractionated amylopectin solution and modified starch solutions. The density of the distilled water and the above prepared solution was determined and recorded. The experimental outcomes were used for the determination of viscosities of the above diluted solutions by using the following formula:

$$\acute{\eta}_2 = \frac{\rho_2 \times t_2}{\rho_1 \times t_1} \times \acute{\eta}_1 \quad (1)$$

where, $\acute{\eta}_1$, $\acute{\eta}_2$, ρ_2, ρ_1, t_1, and t_2 are viscosity of distilled water, viscosity of the test sample, density of the test sample, the density of distilled water, time taken by the water to pass from the upper mark to the lower mark, and time taken by the solutions to pass from the upper mark to the lower mark, respectively.

The relative viscosity of the samples was calculated from the following formula:

$$\acute{\eta}_r = \acute{\eta}_2/\acute{\eta}_1 \quad (2)$$

where, $\acute{\eta}_r$ is the relative viscosity of the test sample

The specific viscosities of the test samples were calculated using the following formula:

$$\acute{\eta}_{sp} = (\acute{\eta}_2 - \acute{\eta}_1)/\acute{\eta}_1 \quad (3)$$

where, $\acute{\eta}_{sp}$ is the specific viscosity of the test sample

The reduced viscosity of the samples was calculated by using the following formula:

$$\acute{\eta}_{red} = (\acute{\eta}_{sp}/C) \quad (4)$$

where, $\acute{\eta}_{sp}$ and C are reduced viscosity of the test sample and concentration of the test samples, respectively.[23,24]

The graph of the reduced viscosity was plotted against the concentration of the readings obtained from the test samples and the same was used for further interpretation of the viscosity studies.

Reducing sugar test

The presence of the reducing sugar in the fractionated amylopectin and modified starch sample was determined. The 1% w/v fractionated amylopectin stock solution and 1% w/v modified starch stock solution were subjected for the reducing sugar test such as Fehling's test, Benedicts test, Tommers test, and Barfoed's test for the determination of the reducing sugar.[25]

Viscosity characterization

The viscosities of the 3% w/v and 6% w/v fractionated amylopectin and modified starch solutions, respectively, were determined using the Brook field viscometer, Model D220. The spindle number 18 was selected for the determination of the viscosity of the test samples. The three readings were taken for reproducible results. The outcome from the viscometer was used to determine the dynamic viscosities (η) of the samples.[26]

Determination of osmotic pressure

The osmotic pressure of 3% w/v and 6% w/v fractionated amylopectin and the modified starch solutions, respectively, was determined by the internal measurement method.[17] The 3% w/v and 6% w/v fractionated amylopectin and the modified starch solutions, respectively, were prepared using 0.9% w/v sterile sodium chloride saline solution aseptically and filtered through the cellulose membrane filter having a pore size of 0.45 μm. The calibrated pipette was attached to the lower end of the funnel, of which the cellulose membrane was fixed tightly without any leakage.

The above fractionated amylopectin and modified starch solutions were filled in the graduated pipette, the whole assembly was inserted in the distilled water [Figure 1], and the equilibrium was allowed to attain within the 24 h. After 24 h, the rise in the level of solution in the calibrated pipette (filled) was measured, and the osmotic pressure of the solutions was calculated in mmHg by the following formula:

$$\pi = 760 \times \left(\frac{T_0}{T_X}\right) \times \left(\frac{B_0}{B_X}\right) - 760 \quad (5)$$

where,

π = Osmotic pressure

T_0 = Length of the pipette covered by the solution before equilibrium

T_X = Length of the pipette covered by the solution after equilibrium

B_0 = Length of the part of the pipette, which is not covered by the solution before equilibrium

B_X = Length of the part of the pipette, which is not covered by the solution after equilibrium.

Determination of enzymatic hydrolysis

The enzymatic degradation of the 3% w/v and 6% w/v fractionated amylopectin and the modified starch solutions with enzyme diastase was studied. The enzymatic hydrolysis study

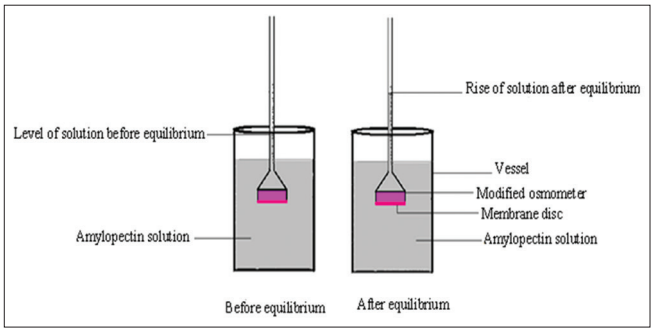

Figure 1: Osmotic pressure measurement by internal measurement method

was used to determine the resistance of fractionated amylopectin and the modified starch to enzyme.[12,16-22,25-27] For the desired optimum activity of the PVE to use, the rate of elimination of the PVEs must be slow. The enzyme amylase present in the blood causes the degradation of amylopectin into smaller fragments. The 3% w/v and 6% w/v fractionated amylopectin and modified starch solutions were prepared as per the determination of osmotic pressure. Fifty milligram of diastase enzyme was added to each solution and the mixture obtained was incubated at 37°C. Ten milliliter of samples was collected after every 10 min interval and analyzed for enzymatic degradation up to 2 h using UV-visible spectrophotometer at wavelengths of 440 and 660 nm.

Interaction with the blood plasma

The change in viscosity of the human blood plasma after the addition of equivalent amount of the formulated PVEs was studied. The Brook Field Viscometer Model D220, model serial number 8496587, with the spindle number 18, was used for the determination of the change in the viscosity of the plasma after the addition of the 3% w/v fractionated amylopectin and 6% w/v modified starch solutions in it separately. All measurements were performed within the torque range of 10–90%. The fresh human plasma was taken for the analysis. Plasma-PVE (1:1) sample solutions of fractionated amylopectin and modified starch were prepared. The three readings were taken for reproducible results.[26]

In vivo study

The pharmacological safety and the effect of fractionated amylopectin and modified starch solutions on the blood coagulation were studied in the female Sprague-Dawley rats as per the CPCSEA, approval number 1344/ac/10/2011-2012. The acute and the chronic toxicity effect of fractionated amylopectin and modified starch solution on the female Sprague-Dawley rats was studied.[28,29]

The 3% w/v and 6% w/v fractionated amylopectin and modified starch solutions were prepared as per the determination of osmotic pressure. The marketed preparation of Voluven, Fresenius–Kabi, AG, and Germany, was used as a standard solution. Adult albino female Sprague-Dawley rats (150–200 g) obtained from the Serum Institute Pune, India, were used for the experiment. They were housed in polypropylene cages with husk

bedding, renewed (every 48 h) under light dark cycle (12:12 h) at around 25 ± 5°C. They were fed with commercial pellet rat chow and water. Four groups of the rats were selected, each including 5 rats. The groups were labeled as a control group, standard group, fractionated amylopectin-treated group, and modified starch-treated group. The control group rats were not given any dose and were kept healthy. The standard group rats were given the marketed preparation containing hydroxyl ethyl starch solution of the Fresenius–Kabi, AG, Germany, the product named Voluven intravenously. The 3% w/v and 6% w/v fractionated amylopectin solution-treated group rats were given fractionated amylopectin solution intravenously and the 3% w/v and 6% w/v modified starch-treated groups were given modified starch solution intravenously. The rats were made hypovolemic by feeding rats with the salt-rich feed and by not feeding them with water for about 24 h. The signs of the hypovolemic shocks were seen in some of the rats. The doses (5 ml/kg of the body weight) were selected for treatment of the rats. The rats were kept under observation after the doses were given to the rats.

About 0.1 ml blood was collected from the retro-orbital route of rats, and the blood coagulation studies were carried out at an interval of 7 days and 1 month to study the acute and the chronic toxicity effect of fractionated amylopectin and modified starch solutions. The blood coagulation studies including bleeding time, partial prothrombin time (PPT), and artificial PPT were performed. The results obtained from the blood coagulation studies of all the four groups were compared after 7 days and after 1 month and they were evaluated.[28,29]

Stability study

The effect of temperature and humidity on the isolated and modified starch of the fractionated amylopectin and the modified starch solutions was studied at a temperature of 25–30°C and at different pH conditions.[2]

1. One gram of the fractionated amylopectin in up to 100 of 0.9% w/v sodium chloride solution to prepare 1% w/v of amylopectin solutions. The clear solution obtained was filtered through the cellulose membrane filter of pore size 0.45 μ. The filtered solutions were collected in the stoppered volumetric flask and were stored at a temperature of 25–30°C
2. One percent weight/volume of modified starch solutions was prepared by diluting the stalk solution up to 1% w/v with 0.9% w/v sodium chloride solution. The clear solution obtained was filtered through the cellulose membrane filter of pore size 0.45 μ. The filtered solutions were collected in the stoppered volumetric flask and were stored at a temperature of 25–30°C
3. One percent weight/volume of amylopectin and 1% w/v of modified starch solutions were dissolved in the buffer solutions (acetate buffer pH of 3.4, 4.0, 5.0, 6.0, and phosphate buffer at a pH of 7.0) in the ratio of 1:1
4. At the regular interval of time (every 1 week), the samples were tested for the change in the appearance of the solution, change in the viscosity, and the chemical stability of the solution.

RESULTS

Isolation of starch

The percentage yield of the isolated potato starch calculated on the basis of the dried tuber weight was found to be 62%, which is near to the previously reported literature (83.5%).[17,18]

Fractionation of isolated starch

The percentage yield of fractionated amylopectin from potato starch calculated on the basis of the dried weight of the isolated starch powder was found to be 75%, which is quite good as compared to the previously reported literature (67%).[17,18]

Physicochemical characterization

The isolated starch from potato showed the satisfactory organoleptic properties of the starch. The iodine test and the spectroscopy test were in compliance with that of the characteristic of the starch. The λ_{max} value of isolated PVE is obtained at 545 nm whereas the reported value as per the literature survey is 540 nm.[17,18] The obtained values of isolated potato starch and fractionated amylopectin for loss on drying are 16.23 ± 0.87 and 11.12 ± 56, which is quite near to the reported values of 10.80 ± 1.55 and 11.60 ± 1.87, respectively. The obtained values of isolated potato starch and fractionated amylopectin for total ash value are 0.52 ± 0.0816 and 0.39 ± 0.0198, which is quite near to the reported values of 0.491 ± 0.05 and 0.35 ± 0.02, respectively. The obtained values of the isolated potato starch and fractionated amylopectin for acid-insoluble ash value are 0.125 ± 0.0647 and 0.070 ± 0.054, which is quite near to the reported values of 0.201 ± 0.003 and 0.22 ± 0.013, respectively.[17,18,21]

The pH of fractionated amylopectin and modified starch solutions was determined using pH meter. The total protein content in the isolated starch samples was determined as per the Indian Pharmacopoeia (I.P.), 2007. Urea was used as the standard sample. For the determination of the protein content, the nitrogen content was determined in the sample by the Kjeldahl method as per the I.P., 2007.[21] The results are summarized in Table 1.

Fourier transform-infrared spectra

The Fourier transform-IR (FT-IR) spectra of the fractionated amylopectin and modified starch samples (as shown in Figures 2 and 3) were found to be similar to that of the spectra of the literature survey. The results are summarized in Table 2.

In vitro characterization

The *in vitro* characterization involved in the determination of the following tests of the fractionated amylopectin and the modified starch solutions by the oxidation and reduction method such as determination of the weight average molecular weight, reducing sugar test, viscosity determination, osmotic pressure determination, enzymatic hydrolysis, and interaction with blood plasma.

Table 1: Physicochemical characterization of isolated starch from potato

Parameter	Result		
	Isolated Potato starch	Fractionated amylopectin	Modified starch solution
Organoleptic test			
Color	White to almost white powder	White powder	Colorless
Odor	Odorless	Odorless	Odorless
Taste	Tasteless	Tasteless	Tasteless
Identification test			
Iodine test	Violet color	Violet color	Violet color
V spectroscopic test	λ_{max} obtained at 545 nm	λ_{max} obtained at 425 nm	λ_{max} obtained at 429 nm
Loss on drying	16.23±0.87	11.12±56	-
Total ash value	0.52±0.0816	0.39±0.0198	-
Acid-insoluble ash value	0.125±0.0647	0.070±0.054	-
Limit test of iron	Passes the test as per the I.P.	Passes the test as per the I.P.	Passes the test as per the I.P.
Oxidative substances	Absent		
pH	7.8±0.012	7.4±0.022	7.2±0.016
Total protein content (µg/g)	0.0016±0.072	0.0007±0.017	0.00644±0.017

I.P.: Indian Pharmacopoeia

Table 2: Wave number (/cm) and structural assignments of the modified starch and fractionated amylopectin from potato starch

Fractionated amylopectin		Modified starch	
Wave number (/cm)	Structural assignments	Wave number (/cm)	Structural assignments
3251.98, 3271.27, 3282.84, 3387, 3452.58	OH-stretch	3529.73, 3321.42	OH-stretch
2929.87	CH-stretch	2929.87	CH-stretch
1546.91, 1425.4	CH_2 bend	1423.47	CH_2-stretch
1163.08	Glycoside COC asymmetric	1165	Glycoside COC asymmetric
1087.85	Coupled CO stretch	1087.85	Coupled CO stretch
927.76	Ring vibration	927.6	Ring vibration
854.47	C_1 group vibration	854.47	C_1 group vibration
763.81	Ring breathing vibration	761.88	Ring breathing vibration
518.85, 572.86, 607.58, 646.15, 667.37	Low frequency ring vibration	700.16, 663.51, 572.86, 518.85	Low frequency ring vibration

Determination of the weight average molecular weight

The weight average molecular weight of the polymer was determined by using the viscosity method using the Mark–Houwink relationship equation.

Intrinsic viscosity and molecular weight–viscosity relationship (Mark–Houwink relationship)

The viscosity of the polymer solution depends on the concentration of the polymer present in the solution.[22] The graph of the reduced viscosity was plotted against the concentration of the readings obtained from the test samples. The linear relationship was established and the extrapolation of the product line to the ordinate gave the intercept, and the value of the intercept was the value of intrinsic viscosity [η]. The weight average molecular weight obtained from the viscosity measurement of the fractionated amylopectin and the modified starch solutions was determined using the Mark–Houwink relationship equation as follows:

$$[\eta] = KM^{\alpha} \quad (6)$$

where,

[η] = Intrinsic viscosity

K and α = Mark–Houwink constant

M = Molecular weight.

The intrinsic viscosities of the fractionated amylopectin and modified starch are 8.388 and 9.181 as mentioned in Figures 4 and and 5 respectively and the molecular weights are 160634.321 g/mole and 149786.124 g/mole, respectively, whereas the molecular weight without fractionation as per the previous studies is reported as 250,000 g/mole.[17,18]

Reducing sugar test

The reducing sugar tests such as Fehling's test, Benedicts test, Tommers test, and Barfoed's test detected the absence of the reducing sugars in the test solution of the fractionated amylopectin and modified starch solutions.

Viscosity characterization

The viscosity of 3% w/v and 6% w/v of the fractionated amylopectin and modified starch solutions, respectively, was determined. The relative viscosity, specific viscosity, and inherent viscosity were computed and are described in Table 3.

Osmotic pressure determination

The osmotic pressure of the fractionated amylopectin solution at 3% w/v and 6% w/v was found to be 28.41 ± 56 and 52.42 ± 68, respectively. The osmotic pressure of the modified starch solution at 3% w/v and 6% w/v was found to be 29.31 ± 12 and 52.98 ± 16, respectively.

Determination of the enzymatic degradation

The enzymatic hydrolysis study was carried out for fractionated amylopectin and modified starch solutions for 90 min. The both compounds were not degraded completely up to 90 min. The results obtained are summarized in Table 4.

Interaction with blood plasma

Plasma-PVE sample solutions of fractionated amylopectin and modified starch in the ratio of 1:1 were prepared. The viscosities of the mixtures are summarized in Table 5. The interaction of the formulated PVEs with the human blood plasma was found to be normal, and no notable change in the viscosities was found.

In vivo study on rats

The pharmacological safety study indicated that the fractionated amylopectin and the modified starch solutions were found to be safe as all the female Sprague-Dawley rats under test after giving the dose were all alive. The blood clotting study results indicated that the fractionated amylopectin and modified starch solutions have the effect on the blood coagulation, but they are in close agreement with the values of control group, and the results were better as compared with the hydroxyl ethyl starch marketed preparation used as standard for the test.[25,28] The results of the *in vivo* study are summarized in Table 6.

Stability study

The storage stability studies were carried out for 6 months. The solutions of the lower pH were not suitable, and the presence of the reducing sugar was observed in the solutions of amylopectin as well as in the solutions of modified starch after 1 month. In addition, the reduction in the viscosity of the solution of amylopectin and oxidized and reduced starch was also observed after 1 month. The no change in the appearance of the solution at the low pH buffer was obtained. Whereas at the neutral pH behavior, the no change in the viscosity and the appearance of the solution was found over the period of 6 months. Furthermore, no considerable changes in the chemical compositions of both the solutions at the neutral buffer pH over the 6 months were observed as shown in Tables 7-11.

DISCUSSION

The starch is a natural polymer, which has the structure similar to glycogen. The starch was isolated from the potato (*S. tuberosum*) tubers. The percentage yield obtained of the starch was satisfactory. The starch obtained was analyzed for the physicochemical characteristics, and the starch isolated was found satisfactory. The isolated starch from potatoes showed the satisfactory organoleptic properties of the starch. The iodine test and the spectroscopy test were in compliance with that of the characteristics of the starch. The values of loss on drying, ash value, and the acid-insoluble ash value were in the compliance as per the I.P., 2007. This indicated that the starch isolated from the potatoes (*S. tuberosum* L. Family *Solanaceae*) was acceptable for the further study.

The starch obtained was fractionated and the amylopectin was isolated from the starch. The fractionated amylopectin was also analyzed for the physicochemical characteristics. The organoleptic characteristics were found in compliance after the physicochemical characterization of the fractionated

Table 3: Viscosity, relative viscosity, specific viscosity, and inherent viscosity of solutions

Rpm	Sample	Viscosity (ή) cP	Relative viscosity ($ή_{rel}$)	Specific viscosity ($ή_{sp}$)	Inherent viscosity
30	3% w/v fractionated amylopectin solution	3.268±0.423	3.2421±0.3417	2.2421±0.3417	0.3747±0.0355
50		3.231±0.331	3.1926±0.2670	2.1926±0.2670	0.3857±0.0279
90		3.156±0.453	3.1185±0.3655	2.1185±0.3655	0.3767±0.0393
30	3% w/v modified starch solution	3.288±0.238	3.2490±0.1919	2.2490±0.1919	0.3921±0.0197
50		3.247±0.269	3.2084±0.2170	2.2084±0.2170	0.3877±0.0226
90		3.226±0.215	3.1876±0.1734	2.1876±0.1734	0.3859±0.0181
30	6% w/v fractionated amylopectin solution	5.796±0.023	5.7272±0.0185	4.7272±0.0185	0.2908±0.0005
50		5.552±0.031	5.4861±0.0249	4.4861±0.0249	0.2836±0.0077
90		5.326±0.012	5.2628±0.0097	4.2628±0.0097	0.2767±0.0003
30	6% w/v modified starch solution	5.812±0.023	5.7430±0.0185	4.7430±0.0185	0.2912±0.0005
50		5.552±0.031	5.4861±0.0249	4.4861±0.0249	0.2836±0.0007
90		5.326±0.012	5.2628±0.0097	4.2628±0.0097	0.2767±0.0003

Table 4: Enzymatic degradation 3% w/v solutions of amylopectin and modified starch

Time (min)	Fractionated amylopectin solution (3% w/v)		Modified starch solution (3% w/v)		Fractionated amylopectin solution (6% w/v)		Modified starch solution (6% w/v)	
	440 nm	660 nm	440 nm	660 nm	440 nm	660 nm	440 nm	660 nm
0	0.9689	0.5452	0.9887	0.4865	1.6692	1.0452	1.9887	0.8865
10	0.9258	0.5012	0.9756	0.4798	1.6268	1.0412	1.9756	0.8789
20	0.8971	0.5824	0.9689	0.4653	1.5971	1.0124	1.9689	0.8634
30	0.8776	0.5651	0.9586	0.4593	1.5776	1.0651	1.9586	0.8556
40	0.8526	0.5426	0.9568	0.4428	1.5536	1.0426	1.9568	0.8482
50	0.8412	0.5217	0.9458	0.4357	1.5421	1.0217	1.9458	0.8375
60	0.8341	0.4958	0.9352	0.4239	1.5314	1.0958	1.9352	0.8293
70	0.7959	0.4444	0.9268	0.4127	1.5219	1.0444	1.9268	0.817
80	0.7612	0.4321	0.9197	0.4091	1.5122	1.0321	1.9197	0.8019
90	0.7444	0.4213	0.9024	0.4003	1.5022	1.0213	1.9024	0.8015

Table 5: Viscosities of plasma and its interaction with the sample solutions

rpm	Sample	Viscosity
30	Plasma	2.578±0.0018
50		2.245±0.0029
100		2.236±0.0022
30	Plasma with fractionated	2.498±0.028
50	amylopectin solution (3% w/v)	2.365±0.019
100		2.261±0.021
30	Plasma with fractionated	2.978±0.021
50	amylopectin solution (6% w/v)	2.295±0.022
100		2.298±0.032
30	Plasma with modified starch	2.5021±0.015
50	solution (3% w/v)	2.4012±0.056
100		2.3112±0.044
30	Plasma with modified starch	3.0251±0.021
50	solution (6% w/v)	2.512±0.022
100		2.452±0.032
30	Plasma with marketed	2.628±0.015
50	preparation (3% w/v)	2.418±0.012
100		2.401±0.025

Table 6: Report of the blood coagulation studies of the marketed preparation, fractionated amylopectin solutions, and modified starch solution with the normal rat blood

Groups	BT (min)	PPT (s)	APPT (s)
Control	3.42±0.87	20.40±0.28	53.92±3.35
Marketed preparation (standard)	4.57±0.68	24.68±0.50	61.35±2.84
Fractionated amylopectin solution	3.52±0.72	20.57±0.63	54.62±2.15
Modified starch solution	3.69±0.42	20.95±0.51	55.64±2.28

BT: Bleeding time, PPT: Partial prothrombin time, APPT: Artificial partial prothrombin time

Table 7: Viscosities of amylopectin and modified starch solutions at pH 7.0 of 1% w/v

Number of days	rpm	Amylopectin solutions			Modified starch solutions
		Potato	Wheat	Maize	
0	30	1.268±0.423	1.365±0.258	1.562±422	1.325±260
3	30	1.267±0.431	1.365±0.258	1.562±422	1.325±260
7	30	1.268±0.425	1.365±0.258	1.562±422	1.325±260
15	30	1.268±0.234	1.365±0.258	1.562±422	1.325±260
30	30	1.268±0.443	1.365±0.258	1.562±422	1.325±260
60	30	1.268±0.473	1.365±0.258	1.562±422	1.325±260
90	30	1.268±0.453	1.365±0.258	1.562±422	1.325±260
120	30	1.268±0.443	1.365±0.258	1.562±422	1.325±260
150	30	1.268±0.413	1.365±0.258	1.562±422	1.325±260
180	30	1.268±0.443	1.365±0.258	1.562±422	1.325±260

amylopectin. The red color obtained with the addition of the iodine solution indicated that the amylose content is very low or the amylose is absent in the sample. The spectroscopic analysis showed the absorption at the λ_{max} 425 and 429 nm, respectively, which indicated that the amylose chain which is responsible for overlapping over the iodine molecule was absent in the fractionated amylopectin samples under examination. Thus, all the parameters studied for the characterization were found to be acceptable for the further study. The pH of fractionated amylopectin and modified

Table 8: Viscosities of amylopectin and modified starch solutions at pH 3.4 of 1% w/v

Number of days	rpm	Amylopectin solutions			Modified starch solutions
		Potato	Wheat	Maize	
0	30	1.268±0.423	1.326±0.156	1.568±360	1.319±154
3	30	1.267±0.431	1.315±0.161	1.555±123	1.309±231
7	30	1.266±0.425	1.225±0.516	1.555±111	1.298±154
15	30	1.266±0.234	1.136±0.426	1.554±112	1.297±157
30	30	1.265±0.443	1.026±0.560	1.436±367	1.279±123
60	30	1.162±0.473	1.015±0.112	1.411±258	1.226±332
90	30	1.058±0.453	0.986±0.164	1.405±117	1.182±214
120	30	1.021±0.443	0.966±0.112	1.368±321	1.115±159
150	30	0.926±0.413	0.936±0.114	1.287±115	1.078±132
180	30	0.887±0.443	0.926±0.612	1.112±451	0.986±121

Table 9: Viscosities of amylopectin and modified starch solutions at pH 4.0 of 1% w/v

Number of days	rpm	Amylopectin solutions			Modified starch solutions
		Potato	Wheat	Maize	
0	30	1.268±0.423	1.326±0.156	1.568±360	1.319±154
3	30	1.267±0.431	1.315±0.161	1.555±123	1.309±231
7	30	1.266±0.425	1.225±0.516	1.555±111	1.298±154
15	30	1.266±0.234	1.136±0.426	1.554±112	1.297±157
30	30	1.265±0.443	1.026±0.560	1.436±367	1.279±123
60	30	1.162±0.473	1.015±0.112	1.411±258	1.226±332
90	30	1.058±0.453	0.986±0.164	1.405±117	1.182±214
120	30	1.021±0.443	0.966±0.112	1.368±321	1.115±159
150	30	0.926±0.413	0.936±0.114	1.287±115	1.078±132
180	30	0.887±0.443	0.926±0.612	1.112±451	0.986±121

Table 10: Viscosities of amylopectin and modified starch solutions at pH 5.0 of 1% w/v

Number of days	rpm	Amylopectin solutions			Modified starch solutions
		Potato	Wheat	Maize	
0	30	1.268±0.423	1.326±0.156	1.568±360	1.319±154
3	30	1.267±0.431	1.315±0.161	1.555±123	1.309±231
7	30	1.266±0.425	1.225±0.516	1.555±111	1.298±154
15	30	1.266±0.234	1.136±0.426	1.554±112	1.297±157
30	30	1.265±0.443	1.026±0.560	1.436±367	1.279±123
60	30	1.162±0.473	1.015±0.112	1.411±258	1.226±332
90	30	1.058±0.453	0.986±0.164	1.405±117	1.182±214
120	30	1.021±0.443	0.966±0.112	1.368±321	1.115±159
150	30	0.926±0.413	0.936±0.114	1.287±115	1.078±132
180	30	0.887±0.443	0.926±0.612	1.112±451	0.986±121

starch solutions was determined using pH meter. The pH of the fractionated amylopectin and modified starch solutions was found to be satisfactory for the use as PVE. The total protein content in the isolated starch samples was determined as per the I.P., 2007. Urea was used as the standard sample. The total protein content determined in the isolated starch was in small quantity, which was found to be satisfactory to use as PVE. For the determination of the protein content, the nitrogen content was determined in the sample by the Kjeldahl method as per the I.P. 2007.

The FT-IR spectra of the fractionated amylopectin and modified starch samples were found to be similar to that of the spectra

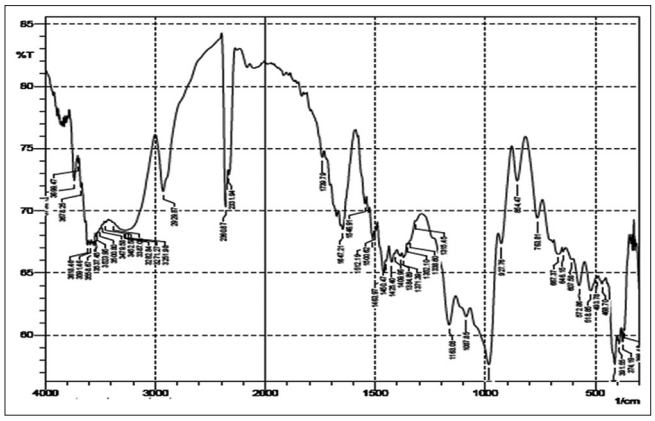

Figure 2: Fourier transform-infrared spectra of the fractionated amylopectin of potato starch

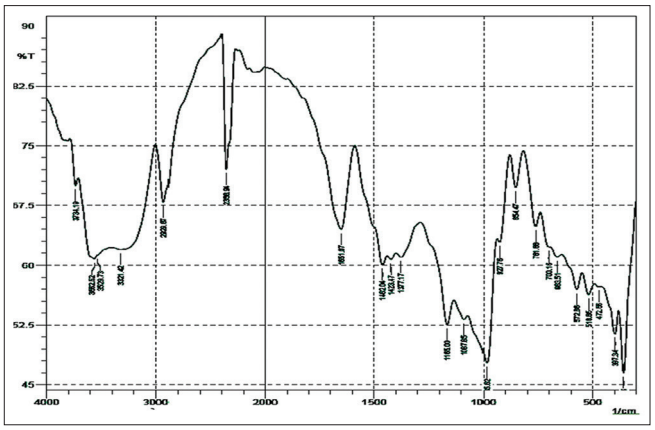

Figure 3: Fourier transform-infrared spectra of the modified starch

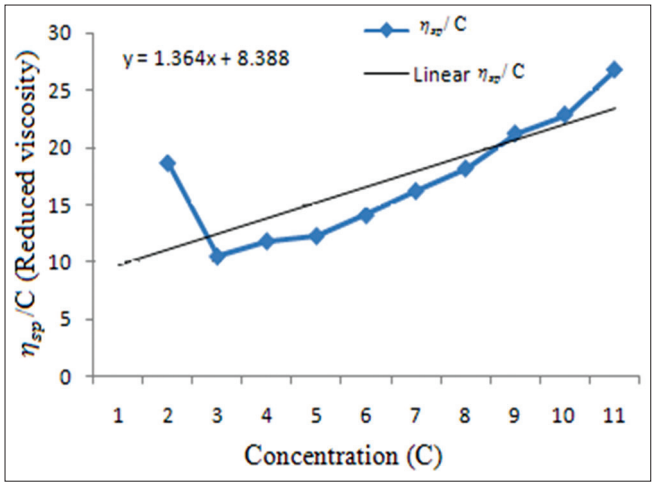

Figure 4: η_{sp}/c (specific viscosity vs. concentration) versus concentration (C) of dilute fractionated amylopectin solution

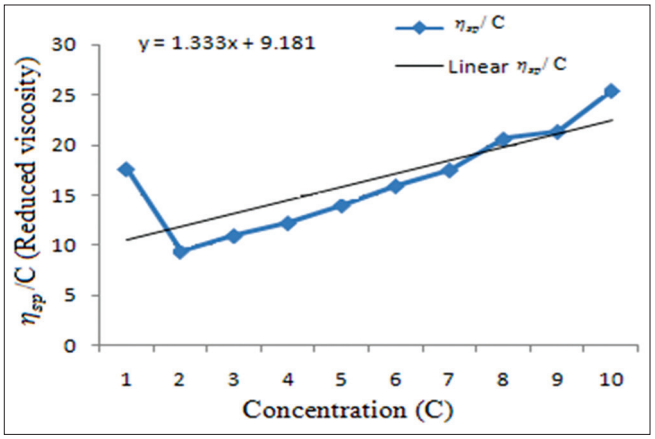

Figure 5: η_{sp}/c (specific viscosity vs. concentration) versus concentration (C) of dilute modified starch solution

Table 11: Viscosities of amylopectin and modified starch solutions at pH 6.0 of 1% w/v

Number of days	rpm	Amylopectin solutions			Modified starch solutions
		Potato	Wheat	Maize	
0	30	1.268±0.423	1.326±0.156	1.568±360	1.319±154
3	30	1.267±0.431	1.315±0.161	1.555±123	1.309±231
7	30	1.266±0.425	1.225±0.516	1.555±111	1.298±154
15	30	1.266±0.234	1.136±0.426	1.554±112	1.297±157
30	30	1.265±0.443	1.026±0.560	1.436±367	1.279±123
60	30	1.162±0.473	1.015±0.112	1.411±258	1.226±332
90	30	1.058±0.453	0.986±0.164	1.405±117	1.182±214
120	30	1.021±0.443	0.966±0.112	1.368±321	1.115±159
150	30	0.926±0.413	0.936±0.114	1.287±115	1.078±132
180	30	0.887±0.443	0.926±0.612	1.112±451	0.986±121

of the literature survey. The *in vitro* characterization involved the determination of the following tests of the fractionated amylopectin and the modified starch by the oxidation and reduction method such as determination of the weight average molecular weight, reducing sugar test, viscosity determination, osmotic pressure determination, enzymatic hydrolysis, and interaction with blood plasma. The weight average molecular weight of the polymer was determined by using the viscosity method. The weight average molecular weight of amylopectin from the potato starch was found to be above 50,000 g/mole, which was satisfactory for the use as PVE. In addition, the molecular weight of the oxidized and reduced starch was found to be satisfactory to be used as a PVE.

The viscosity of the polymer solution depends on the concentration of the polymer present in the solution. The graph of the reduced viscosity was plotted against the concentration of the readings obtained from the test samples. The linear relationship was established and the extrapolation of the product line to the ordinate gave the intercept, and the value of the intercept was the value of intrinsic viscosity [η]. The weight average molecular weight obtained from the viscosity measurement of the fractionated amylopectin and the modified starch solutions was determined using the Mark–Houwink relationship equation $[\eta] = KM^{\alpha}$. The reducing sugar tests such as Fehling's test, Benedicts test, Tommers test, and Barfoed's test detected the absence of the reducing sugars in the test solutions of the fractionated amylopectin and modified starch.

The viscosity of the solutions of the fractionated amylopectin and the modified starch was found close with the viscosity complying with the viscosity of the blood and plasma. The inherent viscosity of the

amylopectin solution was found to be very high, which indicated that the fractionated amylopectin and modified starch prepared have the higher degree of branching. The higher degree of branching may help the molecule to resist against the enzyme amylase. The molecule's resistance would help the molecule to persist for the optimum amount of time into the body to the desired activity, for which the formulation is intended to be given. The viscosity of the marketed preparation 3% w/v solution was similar to the viscosities of 3% w/v solution of amylopectin and modified starch. The osmotic pressure of the fractionated amylopectin and the modified starch solution was determined by the osmometer designed from the dialyzer in the laboratory. The osmotic pressure of the solution depends on the mean molecular weight of the substance present in the solution. The osmotic pressure of fractionated amylopectin and modified starch solutions is shown in Table 4. The normal osmotic pressure of the blood with the normal hematocrit value is 29 mmHg. From the present study, one can predict that osmotic pressure of 3% w/v fractionated amylopectin and modified starch solutions is quite consistent with the normal osmotic pressure of the blood. Six percent of weight/volume fractionated amylopectin and modified starch solutions is indicated to administer in critical condition, which resembles with the literature outcome.

The enzymatic hydrolysis study was carried out for fractionated amylopectin and modified starch solutions for 90 min. During this period, the UV-visible absorbance was found to be decreasing, but the readings were above the baseline taken, which indicated that up to the period of 90 min, the solutions were not converted to the reducing sugar completely. The viscosity of the blood with the normal hematocrit value is in between the value of 3cP and 4cP. The blood plasma has viscosity up to 3cP. The interaction of the formulated PVEs with the human blood plasma was found to be normal and no notable increase or decrease in the viscosities was found. The plasma viscosity should not change as it may create problem in the microcirculation. As no notable change in the plasma viscosities after mixing with the formulated PVE was found, this indicates that the formulated product can be used as the PVE. However, this study is *ex vivo,* and the readings may vary with person-to-person's plasma content.

The pharmacological safety study indicated that the fractionated amylopectin and the modified starch solutions were found to be safe as all the female Sprague-Dawley rats under test after giving the dose were all alive. The blood clotting study results indicated that the fractionated amylopectin and modified starch solutions have the effect on the blood coagulation, but are in close agreement with the values of control group and the results were better as compared with the hydroxyl ethyl starch marketed preparation used as the standard for the test.

CONCLUSION

In the present work, amylopectin was fractionated from the isolated starches of *Solanum tuberosum* and were characterized as per the polymer analysis for the use as plasma volume expander. The osmotic pressure was determined by the modification of the internal measurement method which showed that the osmotic pressure of the 3% w/v and 6% w/v solutions of the fractionated amylopectins and the modified starch were in good agreement to the values obtained from the literature. The enzyme degradation test showed that the fractionated amylopectin and the modified starches have the good resistivity against enzyme. The *in vivo* studies predicted that the fractionated amylopectin solutions and the modified starch solutions were non toxic, but both the solution had the effect on the blood coagulation. The bleeding time, partial prothrombin time and artificial partial prothrombin time were prolonged by the both the test solutions with the minor difference. The storage stability study indicated that the both the solutions were stable at the neutral pH for the period of 6 months. Thus by the characterization of the fractionated amylopectin and the modified starch solution as per the polymer analysis and the *in vivo* study led to the conclusion that both the polymers can be used as the basic material for manufacture of colloidal plasma volume expander

Acknowledgment

The authors would like to acknowledge the Trustees, Bhujbal Knowledge City, MET's Institute of Pharmacy, Adgaon, Nasik, Maharashtra, India, for providing the necessary facilities to carry out this work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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