Impact of a New Formulated Silicone-Based Separator Gel on Clinical Chemistry Assays

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ABSTRACT: *A blood Serum Separator Tube (SST) containing separator gels is widely used for clinical laboratory tests. This study aims to optimize the condition of a newly formulated serum separator gel and to investigate the impact of this gel on some clinical chemistry assays. Design Expert statistical software was used to predict the optimum condition of filler content and to achieve a desired density range. The newly developed polymeric (silicone oil) gel was prepared by using 8 and 10 phr di cumyl peroxide and silica in formulation, respectively. The results reveal that this new gel shows thixotropic behavior with the desired density of 1.05 g/mL. Afterward, a total of 22 clinical assays were performed on samples that were collected in newly developed gel-based serum tubes and commercial SSTs with and without separator gel. In all selected clinical assays, the results of samples collected in SSTs with newly developed gel and commercial gels were more or less the same. Moreover, the experimental data shows this new gel interacts less with blood samples in comparison with commercial gel tubes in some tests. Consequently, the new SST gives satisfactory performance and minimum interference in selected clinical assay results.*

KEYWORDS: *Blood collection tube; Polymeric gel, Silicone oil; Design expert software; Clinical assay.*

INTRODUCTION

A serum Separator Tube (SST) is a useful device for separation and preparation of blood serums for clinical assays. Modern SSTs contain a polymeric gel at the bottom of the tube. This polymeric gel forms a physical barrier between serum and blood cells during centrifugation. There are so many advantages to collecting blood samples in SSTs such as reducing centrifugation time, facilitating storage and transportation of samples, better separation of blood plasma from blood cells, and improvement in serum analyte stability [1-3]. Despite these benefits, using SST is associated with some limitations. The most important issue is the possibility of interaction between separator gel and blood ingredients. Therefore, some studies have been conducted to prepare a new serum separator gel and assess interfering levels of gel ingredients in different clinical chemistry assays. For example, *Sun et al.* [4] prepared a UV-curable thixotropic gel composed of a sorbitol-based gelator in a diacrylate oligomer. They

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investigated the effect of the gel on fourteen different analytes and compared the results with BD serum separator tubes. Emerson [5] provided a separator substance with a polyester backbone that was polymerized in a short time to the desired hardness.

In other studies, *Schouwers et al.* [1] evaluated the effect of polyacryl ester separator gel on the concentration of various analytes in Sarstedt S-Monovette® tubes and observed no significant difference between samples collected in tubes with and without separator gel on the day of phlebotomy. After one day, the statistical differences were observed, but they were not clinically significant. *Bowen et al.* [2] studied the effect of the contents of eight different blood collection tubes on total Free Fatty Acids (FFA), total triiodothyronine (TT_3), and β-hydroxybutyrate (β-HB) concentrations. They concluded that components of the separator gel in SST, reformulated SST and as a result, PPT tubes increase the serum FFA concentration in comparison with other tubes. *Karppi et al.* [6] studied the suitability of three kinds of gel-containing tubes (Vacutainer SST, Vacuette, and Venoject II) for collecting and storing blood samples for Therapeutic Drug Monitoring (TDM). The results showed that these gel tubes were suitable for antiepileptic, antibiotic, asthma, and cardioactive drug measurements, while they were not acceptable for antidepressant drug measurements. *Li et al.* [7] studied the performance of BD SST™ II Plus tubes containing a separator gel compared to BD vacutainer serum glass tubes for special protein testing. *Ercan et al.* [8] investigated the effect of a gel in two different serum separator tubes (Ayset clot activator & Gel tube and BD Vacutainer SST II advance tube) on thyroid tests and compared the result with reference tubes (BD Vacutainer Z tube). In 2019, *Schrapp et al.* [9] examined the impact of two different types of blood tubes (BD PST and Barricor) on the stability of some therapeutic compounds and drugs of abuse in plasma samples.

The main purpose of the present study is to find the optimum amount of fillers in a newly developed gel and to compare the results of some blood tests with commercial blood tubes used in Iran. In order this aim, new formulated silicone-based separator gel is introduced and optimum conditions for the filler content are determined by using Response Surface Methodology (RSM). Also, the performance of the newly developed separator gel in selected clinical chemistry assays is studied. The test results are compared and discussed with those of commercial SSTs without and with separator gels.

THEORETICAL SECTION

Mathematical models are valuable tools for the simulation and optimization of experimental conditions. The statistical models would help to improve operating parameters and the performance of experimental processes. Statistical design of experiments, estimation of coefficients through a mathematical model and prediction of response, and analysis of the model's applicability are three steps used in the experimental design [10].

In the present investigation, Response Surface Methodology (RSM) is applied for the optimization of filler content. One factor design is used for the RSM in the experimental design.

EXPERIMENTAL SECTION

Materials - SST, silicone oil, filler, and chemicals

The material used includes Clot activator tube (tubes with no gel, XINLE clot activator tube, Hebei Xinle Sci & Tech Co. LTD, China), commercial serum separator gel tube (XINLE gel and clot activator tube, Hebei Xinle Sci &Tech Co. LTD, China), silicone oil (SL5300A, Dimethylpolysiloxane, Vinyl terminated, KCC company, Korea), silica (filler, ISATIS silica, Iran), nano-silica (filler, ULTRASIL VN 3, Evonik Industries AG, Germany), Dicumyl peroxide (DCP, High-temperature catalyst, SIGMA ALDRICH, USA).

Preparation of the newly developed gel

Regarding the preparation of the new gel, several experiments were performed to determine the amount of peroxide, curing time, and temperature. The compound with 8 phr (Parts per Hundred Rubber) DCP in formulation with a curing temperature of 160℃ and time of 30 min, was selected due to the appearance of prepared gels. Different amounts of various fillers were added to achieve the proper density for serum separator gel (the detailed process of producing the new polymeric gel is published in the Progress in Rubber, Plastics and Recycling Technology journal [11]).

Optimization of conditions for filler content by Design Expert software

Optimum conditions for filler content were determined using Response Surface Methodology (RSM). The design and the whole extreme values are reported in Table 1.

Table 1: The experimental design layout and corresponding responses.

Run	Factor 1 A: phr	Factor 2 B: Filler	Response 1 Density (g/mL)
1	10	Silica	1.05
2	15	Silica	1.121
3	30	Nano-silica	1.089
4	30	Nano-silica	1.09
5	10	Nano-silica	0.992
6	10	Silica	1.057
7	15	Nano-silica	1.015
8	20	Silica	1.128
9	25	Silica	1.128
10	30	Silica	1.128
11	10	Nano-silica	0.963
12	25	Nano-silica	1.089
13	20	Nano-silica	1.088
14	30	Silica	1.131

Table 2: ANOVA for response surface quadratic model.

Source	Sum of Squares	df	Mean Squares	F Value	p-value Prob > F
Model	0.037	4	9.227E-003	54.82	< 0.0001
A-phr	0.019	1	0.019	114.52	< 0.0001
B-Filler	0.012	1	0.012	73.79	< 0.0001
AB	1. 237E-003	1	1.237E-003	7.35	0.0240
A^2	3.974E-003	1	3.974E-003	23.61	0.0009
Residual	1.515E-003	9	1.638E-004	-	-
Lack of Fit	1.065E-003	5	2.130E-004	1.89	0.2781
Pure Error	4.500E-004	4	1.125E-004	-	-
Cor Total	0.038	13	-	-	-

The fit summary output of Design Expert software suggested that the quadratic model is statistically significant for the density. Tests for determining the significance of the regression model, individual model coefficients, and lack-of-fit must be carried out to obtain a good model. The results obtained from the analysis of variance (ANOVA) demonstrate the validation of the model (Table 2). The R^2 calculated for density was 0.9606 which is reasonably close to 1. The P-value of the density model was obtained at less than 0.0001 which implies the model is significant. The signal-to-noise ratio (a ratio greater than 4 is desirable), which is another important parameter for the model evaluation, was 21.3 in this study. The Lack of fit was insignificant for the response as indicated in Table 2. This also demonstrates that lack of fit is not important compared to pure errors and eliminated terms are unimportant for the models.

The experimental and predicted density are shown in Fig. 1a. The figure demonstrates the proximity of the model prediction with the experimental data and this indicates the validity of the regression models. To check the adequacy of the obtained model, the normal probability versus the plot of the studentized residuals is also demonstrated (Fig. 1b). In this plot, the residual points are located appropriately close to the straight lines and this confirms the normal distribution of errors and the normality of the data.

Table 3: Optimum conditions for filler content.

Number	Filler	phr	Density (g/mL)
1	Silica	10.01	1.05738
2	Silica	10.12	1.05866
3	Silica	10.24	1.06
4	Silica	11.60	1.07462
5	Silica	12.14	1.08003
6	Nano-silica	16.27	1.04513
7	Nano-silica	17.01	1.05142
8	Nano-silica	18.53	1.06302
9	Nano-silica	19.83	1.07141
10	Nano-silica	20.77	1.07663

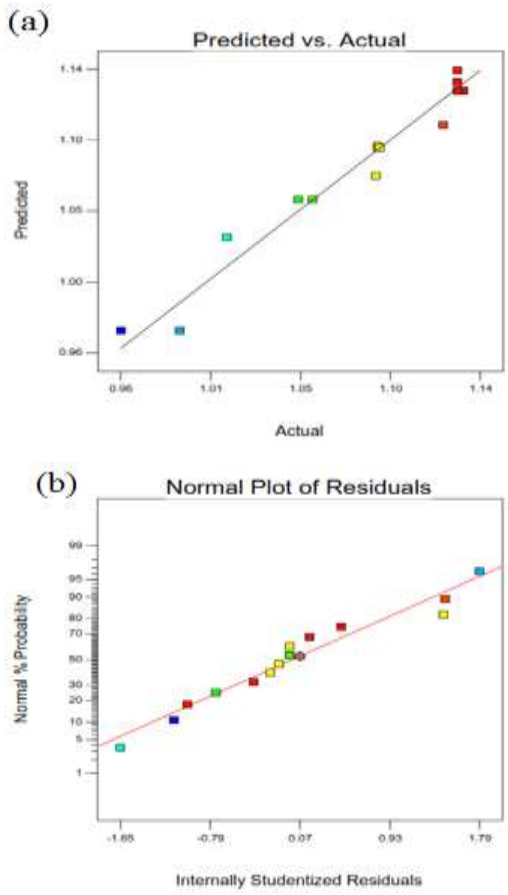

Fig. 1: a) Predicted vs. experimental values, b) Normal probability plots of studentized residuals.

Some optimum conditions for the filler are listed in Table 3. These conditions are provided by the software in order to achieve the desired density range (1.03-1.09 g/mL).

Among the optimization results, by taking economic factors into consideration, the least amount of silica and nano-silica fillers were selected, i.e. 10.01 and 16.27 phr, respectively. Although the compound that was filled with nano-silica had a density in the appropriate range, it could not float after centrifugation and stayed in the bottom of the testing tube. The cured compound filled with 10 phr silica and 8 phr DCP, was chosen as an appropriate gel for the blood serum separator tube due to its suitable density and thixotropy (Fig. 2).

Clinical chemistry assays

In order to estimate the impact of gel on some clinical chemistry assays, about one-quarter of the testing tubes were filled with blood samples, and each experiment was repeated five times then the median value was reported. In total, 22 clinical assays were performed on each blood sample. They included sodium ion (Na), potassium ion (K), calcium (Ca), magnesium (Mg), Total Iron Binding Capacity (TIBC), urea, uric acid, Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), Serum Glutamic Pyruvic Transaminase (SGPT), Creatine PhosphoKinase (CPK), Fasting Blood Sugar (FBS), phosphorus (P), iron ion (Fe), albumin (Alb), creatinine (Cr), Serum Glutamic Oxaloacetic Transaminase (SGOT), alkaline phosphatase (ALP), cholesterol (Chol), triglyceride (Tg), direct bilirubin (B.D) and total bilirubin (B.T).

An auto analyzer (Alpha Classic AT++, Iran) and several reagent kits (Bionik, Iran) were used for running the clinical tests. An auto analyzer is a piece of laboratory equipment that increases the efficiency and the number of performed chemical tests. Alpha Classic AT++ analyzer had 120 cuvette trays with a nominal speed of 280 tests/h. This random access auto analyzer had two separate dispensing arms for reagent and serum [12]. Samples were firstly mixed with particular reagents and subsequently, the chemical reaction products were identified by using a photoelectric detector. Table 4 shows the corresponding wavelengths for the detection of the measured analytes.

RESULTS AND DISCUSSION

The thixotropic gel should be characterized as a stable and chemically inert material. The gel density should be intermediate to that of the blood phases to be separated [13]. The new silicone oil-formulated gel showed thixotropic

Table 4: Corresponding wavelengths for detection of the measured analytes [14, 16, 17, 19, 20, 21-23].

Analytes	Wavelength (nm)
Calcium (Ca)	650
Magnesium (Mg)	600
Total iron-binding capacity (TIBC)	660
Urea	340
Uric Acid	520
Low-density lipoprotein (LDL)	600
Serum glutamic pyruvic transaminase (SGPT)	340
Creatine phosphokinase (CPK)	340
Fasting blood sugar (FBS)	505
Phosphorus (P)	340
Iron (Fe)	562
Albumin (Alb)	630
Creatinine (Cr)	490-500
Serum glutamic oxaloacetic transaminase (SGOT)	340
Alkaline phosphatase (ALP)	410
Total Bilirubin (B.T)	578
Direct Bilirubin (B.D)	546
Cholesterol (Chol)	500
Triglyceride (Tg)	500
High-density lipoprotein (HDL)	600
Potassium (K)	500
Sodium (Na)	420

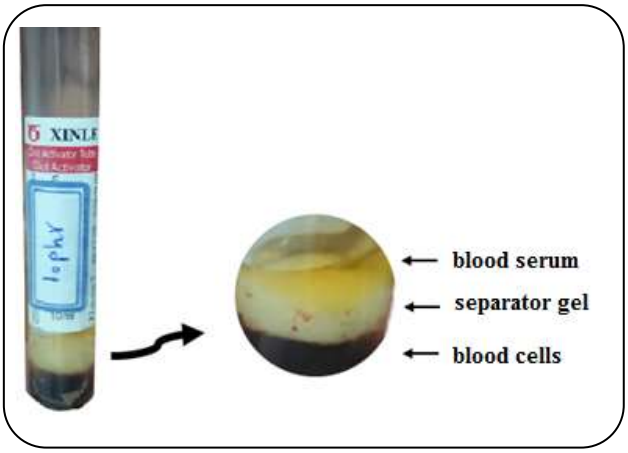

Fig. 2: The appearance of the prepared separator gel.

behavior with the desired density of 1.05 g/mL. This gel moved from the bottom of the tubes during centrifugation and stood between blood phases as shown in Fig. 2. Table 5 compares the test results for twenty-two clinical chemistry assays for the newly developed silicone oil gel SST and commercial SSTs with and without gel.

Calcium concentration

The Arsenazo III Colorimetric method was used for measuring the concentration of calcium in blood serum samples [14, 15]. The calcium concentration for SST with developed gel, and commercial SSTs with and without gel were 9.3, 9.4, and 9.1 mg/dL, respectively (Table 5). Both gels contained tubes that demonstrated higher values in comparison with SST without gel. However, the values for gel-contained SSTs did not have any meaningful differences.

Phosphorus concentration

Conventionally, inorganic phosphorus in serum samples was determined using phosphomolybdate method in which ammonium molybdate reacts with inorganic phosphorus to form a complex that is adsorbed at 340 nm [14]. All measured values in Table 5 were the same with the value of 4 mg/mL. It can be concluded that the existence of the gel in SST did not have any effects on blood serum phosphorus determination.

Potassium ion concentration

In this study, direct spectrophotometric measurement was used for determining potassium ion concentration in serum [16]. The reported values in Table 5 were 3.8 mmol/L for all used SSTs. Similar to phosphorus, the presence of the gel in SST did not result in negative effects on determining blood serum potassium.

Sodium ion concentration

In the colorimetric measurement of sodium, it is precipitated from a protein-free supernatant as the triple salt [17]. The measured values (Table 5) for SST with developed gel, commercial SSTs with and without gel were 138, 138, and 139 mmol/L, respectively, which show identical results for both gels containing SSTs.

Magnesium concentration

Magnesium concentration in serums was measured based on the colorimetric Xylidyl Blue method [18, 19]. The measured magnesium ion concentration for SST with

developed gel, commercial SSTs with and without gel were 1.79, 1.77, and 1.88 mg/dL, respectively. The values for gel content tubes were more or less the same, but they represented higher values for SST without gel.

Iron ion concentration

Serum iron levels can be determined by a specific reaction of ferrous iron with ferrozine [19]. The reported value for newly developed gel content SST was higher (71 µg/dL) when compared with those of commercial SST with and without gel (67 µg/dL).

Total Iron-Binding Capacity (TIBC) concentration

The Serum Total Iron-Binding Capacity (TIBC) measures the total amount of iron that can be bound by serum proteins [20]. The measured TIBC value for newly developed gel SST (313 µg/dL) was close to that of the non-gel commercial SST (314 µg/dL). However, for commercial SST with gel, the measured value was 307 µg/dL which showed a sensitively lower value. In a conclusion, unlike the commercial SST with gel, the SST with newly developed gel did not have meaningful interference.

Albumin concentration

The method used to measure the albumin concentration was the Bromocresol Green method [14]. Table 5 shows the same values (4.6 g/dL) for both gels containing SSTs and a slightly lower value (4.5 g/dL) for SST without gel.

Urea concentration

In this study, Urease-GLDH/UV Kinetic method was used for measuring the concentration of urea in blood serum [14]. The measured values for this analyte were 28, 29, and 29 mg/dL respectively for SST with newly developed gel and commercial SSTs with and without gel. As it can be observed, the value for SST with the newly developed gel is slightly less when compared with the commercial SSTs with and without gel.

Creatinine concentration

Creatinine is a waste product that forms when creatine breaks down and it has been found to form a complex with picric acid under alkaline conditions [19]. The creatinine concentration for SST with developed gel, and commercial SST with and without gel were 0.92, 0.93, and 0.90 mg/dL, respectively (Table 5). Both gel-content tubes demonstrated higher values when compared with SST without gel. However, the values for gel-contained SSTs did not have meaningful differences.

Uric acid concentration

The Enzymatic uricase method was used for determining uric acid concentration in blood serum [14]. The measured values for uric acid for both commercial SST with and without gel were 5.4 mg/dL which was less than that of SST with newly developed gel (5.6 mg/dL).

Aspartate aminotransferase (AST)

In this study, the detection technique of SGOT is Optimized UV kinetic [14]. The AST measured values for both commercial SST with and without gel were 14 U/L which was less than that of the measured value for SST with newly developed gel (18 U/L).

Serum GlutamicPyruvic Transaminase (SGPT)

An optimized UV kinetic method was used for the quantitative estimation of alanine aminotransferase (ALT) in blood serum [14]. The measured values (Table 5) for SST with developed gel, commercial SST with gel, and without gel were 10, 12, and 11 U/L, respectively.

Alkaline phosphatase (ALP) concentration

The determination of serum alkaline phosphatase is based on colorimetric measurement of the formation rate of p-nitrophenol from hydrolysis of para-nitrophenyl phosphate by alkaline phosphatase [19]. Table 5 shows the measured values for SST with developed gel, and commercial SST with gel and without gel as 138, 139, and 141 U/L, respectively. It can be seen that both gels containing SSTs show lower values in comparison with SST without gel.

Direct bilirubin concentration (B.D)

The most common method for measuring bilirubin in serum is the Jendrassik – Gorf method. In this method, diazotized sulfanilic acid reacts with two pyrrole rings of bilirubin [19]. Table 5 shows the measured values for SST with developed gel and commercial SSTs with and without gel as 0.15, 0.17, and 0.19 mg/dL, respectively.

Total bilirubin concentration (B.T)

The total bilirubin concentration in serum was determined by the reaction with diazotized sulphanilic acid in the presence

of caffeine, sodium benzoate, and sodium acetate (Jendrassik – Gorf method) [14]. The measured values for B.T (Table 5) for SST with developed gel and commercial SSTs with and without gel were 1.15, 1.16, and 1.13 mg/dL, respectively. It indicates that both gel-content SSTs show higher values than SST without gel.

Creatine phosphokinase (CPK) concentration

In this study, UV kinetic method was used for the measurement of creatine phosphokinase (CPK) concentration in serum [21]. The CPK values for both gels contained SSTs were the same (71 U/L), while for SST without gel, it was 73 U/L.

Glucose (fasting blood sugar, FBS) concentration

The method for determining glucose concentration in serum was enzymatic (GOD-POD) [14] and the results showed more or less identical values of 187,188 and 186 mg/dL, respectively for SST with developed gel and commercial SSTs with and without gel.

Total cholesterol (Chol) and triglyceride (Tg) concentration

Cholesterol and triglycerides are measured enzymatically in serum or plasma in a series of coupled reactions [22]. Table 5 shows the measured values for SST with developed gel and commercial SSTs with and without gel as 264, 274, and 270 mg/dL for cholesterol and 110, 111, 110 mg/dL for triglyceride, respectively. SST with developed gel and without gel shows the same value (110 mg/dL) for triglyceride. However, for cholesterol, the newly developed gel contained SST shows a lower value when compared with those of commercial SSTs with and without gel.

High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) Cholesterol Concentrations

HDL Is measured directly in serum [22]. For determining LDL concentration, the direct enzymatic colorimetric method was used [23]. The measured values for SST with developed gel and commercial SSTs with and without gel were 34, 37, and 35 mg/dL, respectively, for HDL and 175, 181, 177 mg/dL for LDL. Therefore, the SST with newly developed gel had less interference on the test results of these analytes than that of commercial SST with gel.

Table 5: The laboratory test results for three type serum separator tubes.

Analytes	Tube with new formulated silicone oil-based gel	Commercial serum separator gel tube	Tube without gel
Ca (mg/dL)	9.3	9.4	9.1
P (mg/dL)	4	4	4
Na (mmol/L)	138	138	139
K (mmol/L)	3.8	3.8	3.8
Mg (mg/dL)	1.79	1.77	1.88
Fe (μg/dL)	71	67	67
TIBC (μg/dL)	313	307	314
Alb (g/dL)	4.6	4.6	4.5
Urea (mg/dL)	28	29	29
Cr (mg/dL)	0.92	0.93	0.9
Uric Acid (mg/dL)	5.6	5.4	5.4
SGOT (U/L)	18	14	14
SGPT (U/L)	10	12	11
ALP(U/L)	138	139	141
B.T (mg/dL)	1.15	1.16	1.13
B.D (mg/dL)	0.15	0.17	0.19
CPK (U/L)	71	71	73
FBS (mg/dL)	187	188	186
Chol (mg/dL)	264	274	270
Tg (mg/dL)	110	111	110
HDL (mg/dL)	34	37	35
LDL (mg/dL)	175	181	177

CONCLUSIONS

The aim of this study was to optimize the filler content of a newly formulated silicone-based separator gel by using Design Expert software in order to achieve the desired density range (1.03-1.09 g/mL) and compare the performance of the new gel with some commonly used SSTs. Among the optimum conditions provided by the software in order to achieve the desired density range, the least amount of silica and nano-silica fillers (respectively 10.01 and 16.27 phr) were selected. Despite the density of the compound filled with nano-silica being in the appropriate range, it did not show thixotropic behavior. The cured compound filled with 10 phr silica had suitable density (1.05 g/mL.) and thixotropy, therefore this compound (containing 10 phr silica and 8 phr DCP) was selected as an appropriate gel for the blood serum separator tube.

When the newly formulated gel was developed, its performance was compared to some commercial serum separator gel tubes used in Iran (XINLE gel and clot activator tube) and clot activator tube (tube with no gel) for twenty-two different analytes. In all 22 clinical assays, the results of samples collected in SST with the newly developed gel and commercial gel content SSTs were more or less the same. The SST with newly formulated gel had less interference in test results for LDL and HDL in comparison with the value for commercial SST with gel. Therefore, it seems that the SST with newly developed gel represented an acceptable performance and interference in selected clinical assay results. It is worthy of note that more detailed clinical analyses are needed before launching this product.

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