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Zeta Potential Optimization of Nano Chitason/$SrCl_2$/MgO Suspension for Electrophoretic Deposition Using Taguchi Method

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ABSTRACT

The stability of Electro Phoretic Deposition (EPD) suspensions containing nanoparticles relies on the impact of Zeta Potential (ZP or ζ). This property ensures that the nanoparticles have a consistent and stable surface charge, resulting in a uniform and stable coating. This research has been conducted as an experimental study and used the Taguchi method to design experiment optimization of the Zeta potential values, which were obtained by preparing nine suspensions. The study aimed to determine the optimal ZP value for the EPD suspension created with three materials mixed: nanochitason, Chitason/$SrCl_2$/MgO, and a constant value of hydroxyapatite (HA) with consideration of the pH effect. After conducting an analysis, it was found that the suspension's Zeta Potential is negatively charged below a pH value of 8.22. Between 8.22 and 9.7, the ZP has a positive charge. The suspension's isoelectric point (IEP) is 8.22, with a high correlation coefficient indicating the model's reliability in predicting responses. The analysis showed that $SrCl_2$ has the most significant impact on the suspension's ZP, followed by Chitason (CH), with MgO having the least impact. The results demonstrate the effectiveness of this analysis in determining the optimum ZP value for various solutions prepared from different biomaterial particles.

1. Introduction

One of the most significant current discussions in the biomaterials coating process is Electro Phreatic Deposition (EPD); through the process of EPD, charged particles in a stable colloidal suspension are propelled through a liquid by an electric field and deposited on a conductive substrate that is negatively charged to create the desired coating layer [1]. This technique has been widely utilized in orthopedics and Osseointegration because of its ability to apply different coating materials, such as organic or non-organic, or mixed in the form of suspension. Recently, researchers have shown an increased interest in 316L stainless steel alloy, which is widely used as a biomaterial for implantation due to its properties, such as biocompatibility and high strength. It is important to consider that the alloy may be surrounded by fibrous tissue, potentially releasing toxic ions into the body from its surface. This could lead to the implant becoming loose and moving within the b [2]. Bioactive materials have been applied to the metal implant surface as a workable fix for these issues. Such bioactive layers are distinguished by their ability to promote metal surface osteointegration as well as by their capability to decrease or even suppress the ions released from the metal; in this case, the implant process will last for a long time in the human body without any harmful effect

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for the host. Numerous bioactive materials have been applied to the metal as coatings, such as Chitosan (Ch), Strontium Chloride ($SrCl_2$), Magnesium Oxide (MgO), and Hydroxyapatite (HA), Xiong, et al.[3, Afifi, El-Naggar, et al.[4]. Some studies have enhanced our understanding of the effect of Zeta Potential, which indicates EPD suspension stability that contains nanoparticles. The surface charge of the nanoparticles becomes more stable. It provides a uniform and stable coating layer because particles will agglomerate if the ZP has a value of less than 22 mV [3, 4]. The importance of the ZP was determined by [5], who did an investigation which was aimed at improving the compositions of liposomes prepared by a thin-film wetting technique for three variables which were phosphatidylcholine, cholesterol, stearyl amine, and an input for the DOE analysis. While Vesicle Size, Polydispersity Index, and Zeta Potential were the response of the optimizing model, it is worth mentioning that the study considered two values of the suspension pH. From the study, it has been shown that the analysis was able to determine the effect of each component of each material on the zeta potential value. The optimized compositions provided highly stable systems. DOE factorial design was used by [6] for EPD parameters which were coating material concentration, sedimentation time, applied current, suspension pH, and polymeric dispersant concentration studied on the sedimentation efficiency. The deposition rate of the coating materials was analyzed using a 23-factor design technique for three independent variables; the concentration of the coating material in the bath, the current, and the time. In 2017 [9], the Taguchi method could determine the optimal voltage, time, concentration, and temperature parameters of the EPD process. Chitosan and a mixture of chitosan and hydroxyapatite (HA) were used as a coating suspension, and 316L stainless steel was considered a substrate. The proposed analyses found that the optimum conditions for precipitation of the chitosan and HA layers could be predicted: 50 V, 5 min, 3 g pH/L, and 30 °C. Although electrophoretic light scattering (ELS) is frequently used in laboratories, no validated protocols have yet been suggested. In a study conducted by [7], DOE with ANOVA analysis was used to standardize a protocol for ELS-based zeta potential measurements of nanomaterials and a method to achieve these measurements. The work considered several variables that might affect the zeta potential measurement results. Among the factors considered were the sample temperature, the type and batch of measurement cells, and the analyst's confidence in the protocol's reliability. From this work, it has been determined that the proposed protocol was judged to be reliable, precise, and consistent with ISO standards. Nanomaterials with negative and positive charges had protocol uncertainty of 14 and 12 percent, respectively, demonstrating the dependability of the results and approving the validity of the used protocol. The Taguchi DoE approach optimizes the EPD process to obtain coatings with maximum precipitation yield and minimum standard deviation [8]. Taguchi's Design of Experiments (DoE) approach optimizes EPD parameters such as applied voltage, electrode spacing, and deposition time. A "uniform" coating was obtained using a deposition time of 7 min and a deposition voltage of 20 V. Silver-doped hydroxyapatite (AgSr-HA)/chitosan composite coatings were deposited on 316L stainless steel (SS) substrates by electrophoresis deposition (EPD). and a concentration of 5 g/L AgSr-HA particles in suspension. Properly applying statistical models can decrease the cost of producing expensive polymeric nanoparticles. The statistical models and the physicochemical properties of the drug molecule in the formulation process were considered in the study conducted by [9]. The formulation has numerous uses, such as drug-targeting sustained-release therapy and implants. The study's objectives were to identify a wide range of crucial process characteristics necessary for producing high-quality products and to optimize the parameters, focusing on the safe clinical use of drug products. Zeta potential, the particle size of the Nanoparticles, and the composition of the Nanomaterials were considered in this study. Significant parameter correlations were positively evaluated and identified using statistical design to prepare and produce the desired nanoparticles. The study also determined the direct or indirect influenced correlation of significant parameters. A Study by [10] has found that Taguchi DoE could use to optimize the EPD process, which was utilized for orthopedic requests. The optimization process was conducted for suspension concentration and pH, as well as the EPD process parameters, which include the cell voltage, time of coating, and electrode distance. The substrate was 316L SS, and the coating suspension consisted of chitosan/gelatine/Cu-BG(Boa-active Glass). Taguchi array of L25 type was considered for the DOE design. The results obtained from the study suggested that the Taguchi DoE approach has been approved to be a valuable tool for optimizing with a high level of accuracy would be optioned since it could minimize experimental trailers. DOE was considered an optimization tool for drug delivery systems for the brain. Poly(d,l-lactic-co-glycolic) (PLGA) as nanoparticles, several Nano formulations were considered an input variable, and the ZP was the output response. From the study, it has been concluded that this approach would provide optimal properties for the drug co-delivery system with different formations. DOE was considered an optimization tool for drug delivery systems for the brain, such as the work of [11] on how to investigate the Physical and chemical properties of Poly(d,l-lactic-co-glycolic) (PLGA) as nanoparticles, several Nano formulations were considered as an input variable, and the ZP was the output response. The study concluded that this approach would provide an optimal property for the drug co-delivery system with different formations. Another study [12] examined the factors controlling the physical properties of nano gels using a single drug. Then they investigated whether these models could be applied more generally to various drugs. An experimental design approach (DOE) was used to investigate nanoparticle size, ZP, and encapsulation efficiency to optimize formulations by systematically determining the effects of and interactions between nano gel formulations and drug delivery parameters. Three formulation factors were selected: chitosan concentration, the ratio of chitosan to cross-linker, sodium triphosphate, and chitosan to the drug. [13] Utilized the design of the experiments using the Taguchi method to select the optimal conditions (suspension effort, time, and concentration). that give the best results. For the deposition of dense YSZ overlay on YSZ plasma coating by EPD under different process parameters, the best case of coating thickness was at 40 V, 5 min, and ten g/L. Therefore, the reasons these constructs were studied before and how the environment was chosen are critical for further exploitation of the results. Finding the most suitable composition for the specific purpose and achieving the best results by finding the best suitable mixtures with an acceptable zeta potential to conduct for coating the ss316l samples was one of the challenges this time. In general, as observed from prior studies, there are quite a few research studies on EPD suspension optimization based on ZP. It is clearly seen that most of the previous work focused on the EPD parameters optimization rather than suspension optimization. Therefore, another motivation for this study is to select the best ZP of the EPD suspension using the Taguchi method considering the suspension pH effect and particle size. The outcomes of this research will be utilized for the subsequent work conducted by the authors on these nanomaterials and their concentration.

1. Experimental procedures

1.1 Suspension materials

In the current study, the EDP suspension was made from these flowing materials; Chitosan (CH), Strontium Chloride ($SrCl_2$), Magnesium Oxide (MgO), and Hydroxyapatite (HA). Nine suspension samples of these materials were mixed in a 25-beaker. Use ethanol (99.9%), citric acid (vinegar) (3 drops per beaker), and distilled water (6 drops) as solvents. This research involved using materials with varying particle sizes, ranging from 20-50 Nano-meter, and a purity level of 99% for all powders. The CH, HA, and MgO nanopowder were procured from Sigma Aldrich in Germany, whereas $SrCl_2$ was obtained from Chongqing Yuanhe Fine Chemical Co. Ltd in China. The weight percentage of 9 samples is conducted in the Laboratory of Materials Engineering, College of Engineering, Diyala University. These samples were each placed on a stirring machine for 15 minutes to thoroughly mix the components and on a digital ultrasonic cleaner for half an hour to agitate the nanoparticles.

1.2 Experimental design

After an intensive literature review that was conducted, it has been found that the main influencing factor affecting the EPD process is the zeta potential. Zeta potential is considered a crucial factor that determines both the repulsion and attraction between suspension particles, and it is also considered the vital indication factor for the dispersion stability of the suspension. It has been well established that the zeta value is a useful indicator for particle surface charge in the EPD suspension; this value parameter is mainly associated with the composition of the suspension materials. Other important response functions are mobility (μ) and pH.

The zeta value of nanoparticle suspensions is influenced by their composition and the environment in which they exist. It is crucial to attain high ZP values, which can be either positively or negatively charged to prevent particle aggregation. The surface charge of suspension is a critical factor in determining their electrostatic interaction with bioactive substances and their interactions during the EPD process. [14]. In this work, the L9 factorial fraction design has been utilized using Taguchi DoE, a MINITAB 17™ statistical software used to analyze the data. As shown in **Error! Reference source not found.**, these samples will be used to select the best suspension from these mixtures of materials by using DOE as an optimization process. In this analysis, the Independent variables have been systematically ranged at 3 levels with nine runs, as shown in **Error! Reference source not found.**. It is worth mentioning that HA was kept at a constant value of 4%, as suggested by previous works [15, 16]. The outcome of this factorial analysis is to determine the best ZP value with the highest value range, as the previous literature recommends.

Table 1: Composition of the samples used for the preparation of the design of experimental as input variables; presented in the weight percentage.

Run No.	Ch	$SrCl_2$	MgO	HA
1	0.5	0.01	1	4
2	0.5	0.50	3	4
3	0.5	1.00	5	4
4	1.0	0.01	3	4
5	1.0	0.50	5	4
6	1.0	1.00	1	4
7	1.5	0.01	5	4
8	1.5	0.50	1	4
9	1.5	1.00	3	4

1.3 Characterization of the EPD suspension

The most important test used to characterize the suspension is measuring the ZP value for each run sample. The stability of the suspension conducted in this measurement was measured by Zeta-Plus (Zeta Potential Analyzer) technique. To ensure precise measurements, the solid content in all suspensions was diluted to 0.1 g/L. This process was at the Nanotechnology and advance materials research center, University of Technology, Iraq. Furthermore, the particle mobility, particle size of the suspension, and pH values were also evaluated. Composition (Wt%) with independent variables were Ch= 0.5, 1 and 1.5/ $SrCl_2$ = 0.01, 0.50, and 1.00, and MgO = 1, 3, and 5.

2. Result and discussion

2.1 Preliminary results of the suspension

An experimental examination for the nine runs was achieved to obtain the Zeta, particle mobility, and pH values. The obtained data are shown in Table 2; it can be seen clearly that the zeta values were in the approximate range of 22 to 75 mV. Also, the particle mobility value was between 1.7 to 5.7 µm s^{-1}. $V^{-1.}$ cm.

On the other hand, the pH value was above 7 and less than 10, which means the trend of the pH of these suspensions group is in the alkaline range, which indicates the effect of the composition of the primary candidate materials considered in this study. It is worth mentioning that pH strongly affects the coating morphology of the EPD process [17]. As mentioned earlier in the previous section, the materials used were Ch which has a 7.4 pH value, $SrCl_2$ value is 6.5, and MgO has a pH value of 10-12, which means all these combinations are alkaline. **Error! Reference source not found.** shows that the highest pH value (more than 8) was obtained for the suspensions with MgO values of 3 and 5, respectively. It is not surprising to see this range of pH of these combination materials, as well known that $SrCl_2$ can be easily dissolved in the suspension with water base to it ions, and Sr ions is an alkaline ion [18]. It is clear that in this electrolyte suspension, HA was considered with these 9 runs, and it can raise the electrolyte's pH due to CaP ions that release in the electrolyte. It has been reported that HA could increase the solubility of Ch, which is difficult to dissolve in alkaline conditions. These results agreed with the published literature [2].

It has been well established that ZP is considered an indication of EPD suspension stability that contains nanoparticles; with a ZP value of more than 25 mV, the surface charge of the nanoparticles becomes more stable and would provide a uniform stable coating layer because particles will agglomerate [3, 4].

One of the exciting results that could be obtained from this **Error! Reference source not found.** is that the lowest value of ZP was -22.64 at pH value of 9.9, which means the electrolyte becomes highly alkaline, and this would cause difficulty in the solubility of the CH due to a reduction in the electrostatic charge of the particle repulsion. It has been seen that this run has the lowest value of mobility; this was agreed with previous work conducted by [3].

From the table, it can be noticed that with a Ch value 1% and MgO 5%, two positive charges were seen with a value of +69.14 and +67.70, respectively; it can be argued this to the fact that has been reported by previous literature that provides that CH can provide a positive charge of the Zeta [10]. On the other hand, the highest negative value of the zeta potential of the electrolyte was -75.31; this has Ch value of 1% also, but a value of 3% for the MgO.

Fig.1 presents the relationship between ZP and suspension pH; the zero ZP refers to the isoelectric point (IEP). IEP is the pH at which the zeta potential is zero, and it records the zeta potential as a function of pH. It has been approved that the surface nanoparticles' properties are attributed to the solution's pH value [19]. From Table 2, it can be expected that below pH value of 8.22, the ZP is negatively charged, while between 8.22 and 9.7 the ZP has a positive charge. Moreover, the IEP value for this combination of materials that formed these particular suspensions is 8.22. It could have been concluded that this graph would provide an understating of ZP behavior for this alkaline aqueous medium at various pH. In brief, the general criteria between stable and unstable suspensions are +30 and/or -30 mV. Particles with ZP higher than +30 mV or more negative than -30 mV are generally stable. In current work, the highest value of ZP were at runs number four and five. Fig. 1A and B shows the relation between ZP and mobility with pH at different experimental runs, respectively. It is clear in Fig. 1A that at lower pH, particles were negatively charged, and the surface charge changed from negative to positive as the pH increased. The suspension is most stable at pHs between 6.5 to 8, and 9 to 9.5. From Fig. 1B, it can be seen that values of mobility are higher in alkaline regions than in acidic ones.

Table 2: The L9 fractional factorial design produced responses based on experimentally measured values of ZP, particle mobility, and pH for nine different

Run no.	**Ch (%)**	**SrCl$_2$ (%)**	**MgO (%)**	**Zeta (mV)**	**$\mu\times10^{-8}$ ($m^2\ s^{-1}.\ V^{-1}$)**	**pH**
1	0.5	0.01	1	-44	3.2	7.29
2	0.5	0.5	3	-32	2	7.48
3	0.5	1	5	-32.3	2.48	7.56
4	1	0.01	3	-75.31	5.78	8.22
5	1	0.5	5	69.14	5.3	8.69
6	1	1	1	-34.03	2.61	7.32
7	1.5	0.01	5	67.7	5.19	9.22
8	1.5	0.5	1	-35.66	2.74	6.41
9	1.5	1	3	-22.64	1.74	9.91

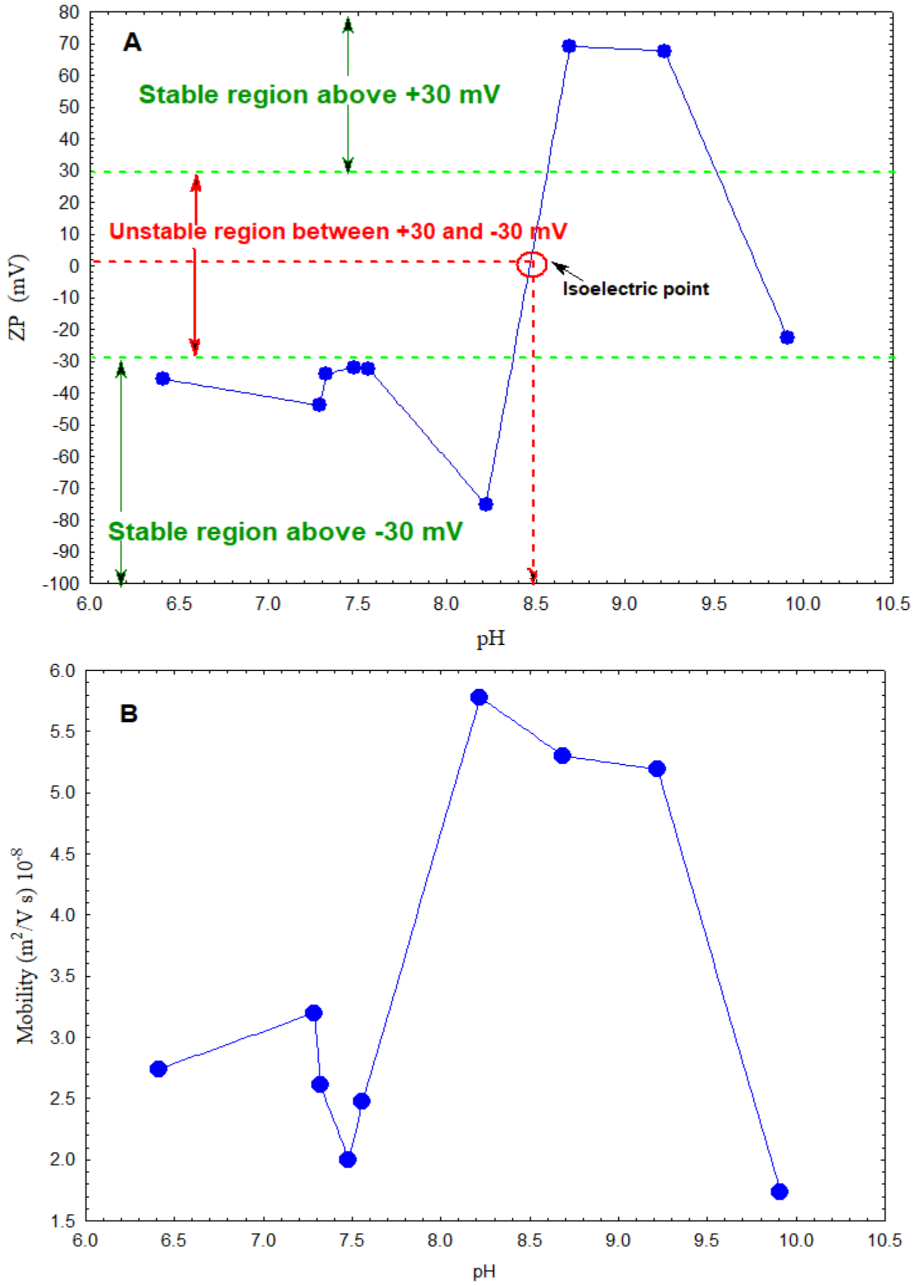


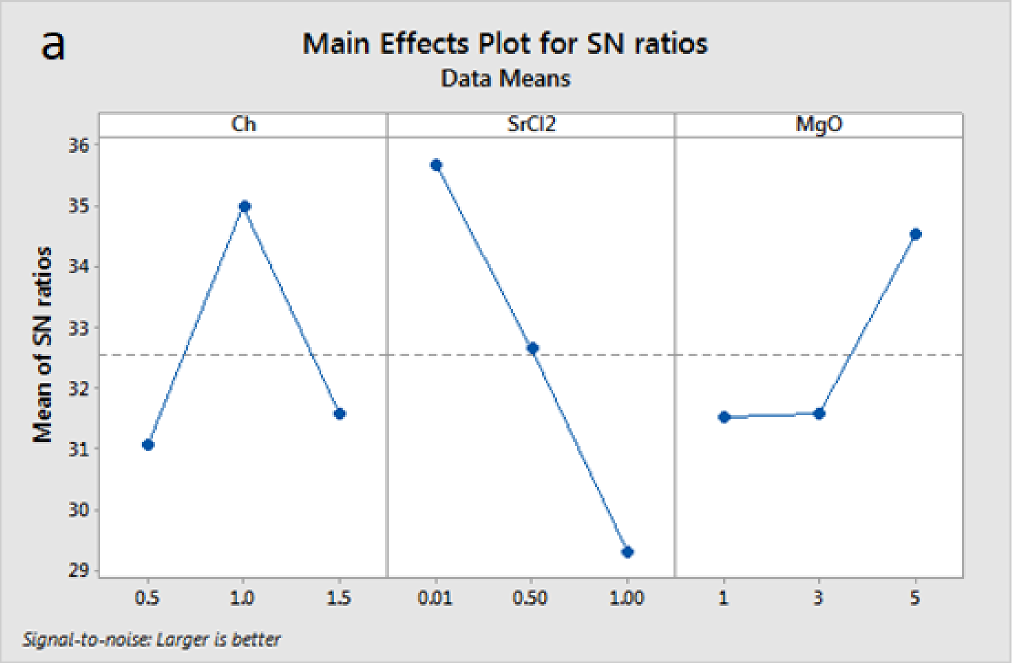
Figure 1. Relationship between (A) ZP and pH and (B) mobility and pH as a function of each run

2.2 Zeta potential, mobility, and pH optimization

An in-depth literature review has been conducted on the zeta potential and its effect on the EPD process by considering different nanoparticles for preparing the EPD solution. After a thorough explanation was shown in the previous section for L9 fractional DOE design, the 9 runs with different concertation were set as an input, and the output value for this factorial design was ZP. After the results were analyzed using Minitab 17 software. In **Error! Reference source not found.**, the response of signal-to-noise ratios for considering material was presented, and it showed the levels of the CH, $SrCl_2$, and MgO with their rank according to analysis. It has been determined that $SrCl_2$ has ranked as one, which means it is a more significant impact on the suspension ZP, followed by Ch., where MgO was ranked as a third value that has less impact on the ZP of the suspension.

The S/N ratio response graph for the ZP and solution composition is presented in Fig. 2a, further confirmed by the analysis results in **Error! Reference source not found.**. A good performance would be obtained when the S/N ratio has a higher value to determine the optimum level of the factors.

After transforming experimental values into a signal-to-noise ratio (S/N), Taguchi analysis uses the signal to represent desired output characteristics (mean) and noise to represent undesired output characteristics (standard deviation) [20]. As shown in Fig. 2b, the proposed models are also satisfactory since all residuals follow a linear pattern, as reported by.[21]. Regarding the analysis of the Residuals - normal probability plots of a predicted response for ZP and solution composition shown in Fig. 2b. From the figure, it can be noticed that the obtained results from this analysis have a uniform pattern within the straight line; this would provide a reasonable indication for the model prediction as it has been reported by previous literature [22]. The most striking result from the data is that the proposed analysis could be considered a useful tool for determining the optimum ZP value from different solutions prepared from different biomaterials particles.



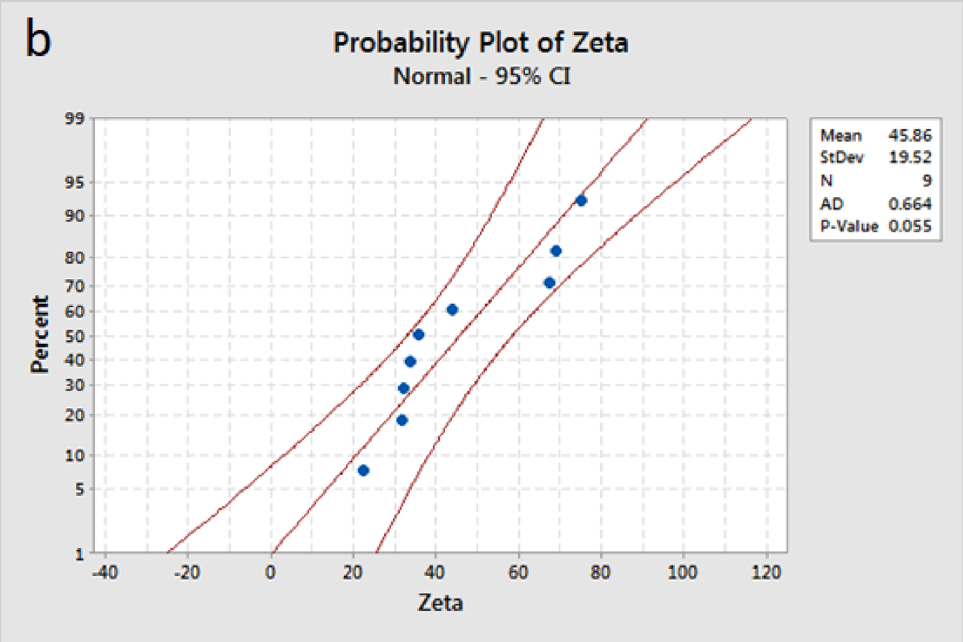


Figure 2. (a) S/N ratio response graph for the ZP and solution composition

2.3 Regression and statistical analysis

The mathematical and statistical analysis represents a powerful tool to correlate responses (dependent variables) to independent variables. In the current work, ZP, mobility, and pH are the dependent variables, while CH, $SrCl_2$, and MgO are the independent variables. A linear model with interaction is suggested to correlate the variables. Statistica software version 10 was used during regression and statistical studies. 1000 was the number of iterations, 1×10^{-6} was the convergence criterion, and Levenberg-Marquardt was the estimation method. The model structure was according to Eq. 1.

$$Y = a_o + a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 \quad (1)$$

Where Y represents the dependent variable $(\zeta, \mu, and\ pH)$, x_1, x_2, and x_3 represent the independent variables (CH, $SrCl_2$, and MgO), a_o is the absolute term, a_1, a_2, and a_3 are the individual-effect term, and a_{12}, a_{13}, and a_{23} are the interaction-effect term. After regression, three responses were obtained with 0.901, 0.902, and 0.963 correlation coefficients (R^2), respectively (Eqs. 2, 3, and 4).

$$\zeta = -42.26 - 41.24\mathrm{C_{CH}} + 61.29\mathrm{C_{SrCl_2}} - 8.96\mathrm{C_{Mgo}} - 25.23\mathrm{C_{CH}C_{SrCl_2}} + 28.89\mathrm{C_{CH}C_{Mgo}} - 6.57\mathrm{C_{MgO}C_{SrCl_2}} \quad (2)$$

$$\mu = 1.13\times10^{-8} + 1.06\times10^{-8}\mathrm{C_{CH}} - 2.24\times10^{-8}\mathrm{C_{SrCl_2}} + 1.72\times10^{-8}\mathrm{C_{MgO}} + 1.86\times10^{-8}\mathrm{C_{CH}C_{SrCl_2}} - 0.76\times10^{-8}\mathrm{C_{CH}C_{MgO}} - 0.95\times10^{-8}\mathrm{C_{MgO}C_{SrCl_2}} \quad (3)$$

$$\mathrm{pH} = 8.45 - 2.68\mathrm{C_{CH}} - 2.4\mathrm{C_{SrCl_2}} - 0.007\mathrm{C_{MgO}} + 3.4\mathrm{C_{Ch}C_{SrCl_2}} + 0.64\mathrm{C_{CH}C_{MgO}} - 0.11\mathrm{C_{MgO}C_{SrCl_2}} \quad (4)$$

Eqs. 2, 3, and 4 represented the predicted data. Fig. 3 shows the predicted response variables against the experimental one. It is clear that the results were close, and the equations act as an effective tool for data representation. Eq. 2 shows the effect of blend concentrations on the zeta potential. The coefficient of $SrCl_2$ was the highest, followed by CH and MgO, which agree with Taguchi results**.**

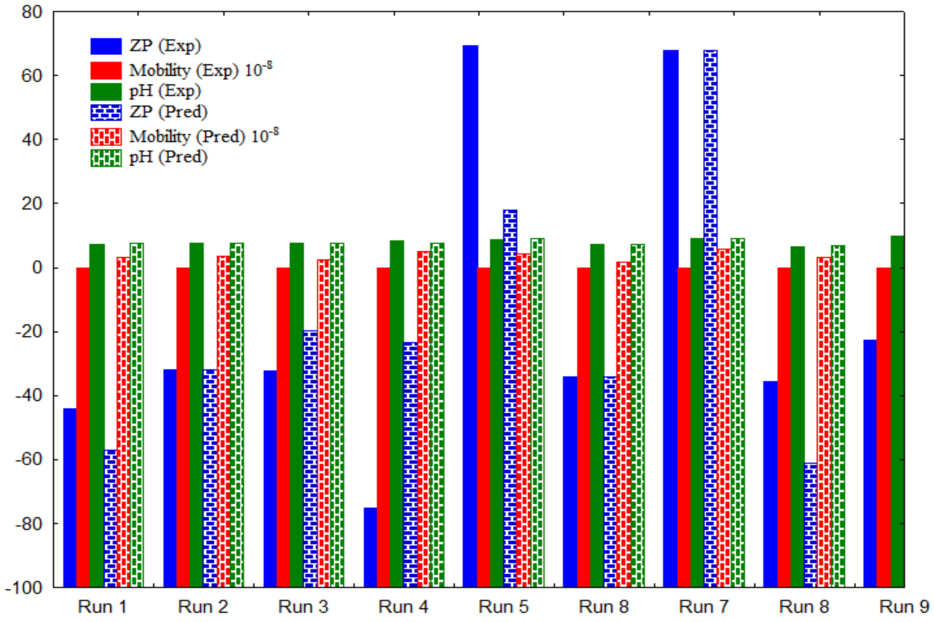


Figure. 3 Predicated response variables against the experimental one

3. Conclusion

In line with the experimental data and optimization analysis, some conclusions can be drawn as follows:

- According to the collected data, suspensions containing MgO values of 3 and 5 had the highest pH level (greater than 8).
- The study concluded that at a pH of 9.9, the value of ZP was -22.64, indicating that the electrolyte becomes highly alkaline. This can lead to a reduction in the electrostatic charge of the particle repulsion, causing difficulty in the solubility of Ch.
- The Ch value 1% and MgO 5% measurements indicated two positive charges with values of +69.14 and +67.70, respectively. This is due to Ch providing a positive charge to the Zeta. The ZP has a negative charge below a pH value of 8.22, but between 8.22 and 9.7, it has a positive charge.
- The IEP value for this specific combination of materials in the suspensions is 8.22. This provides insight into the behavior of the ZP in this alkaline aqueous medium at different levels. pH.
- The response of signal-to-noise ratios for considering material showed the Ch, $SrCl_2$, and MgO levels with their rank according to analysis. It could be concluded that $SrCl_2$ has ranked as one, which means it is a more significant impact on the suspension ZP, followed by Ch., where MgO was ranked as a third value that has less impact on the ZP of the suspension.

According to the Taguchi Analysis, the SN ratios revealed a high R-sq value of 99.3%, implying that the suspension composition's responses can be reasonably predicted.

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