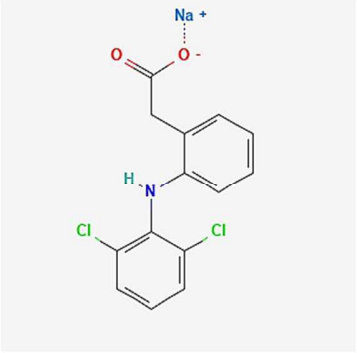
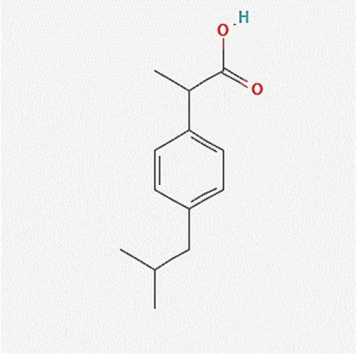
Chapter 3
Materials and methodology

3. Materials and methodology

3.1 Materials

Diclofenac sodium (DCF) and Ibuprofen (IBU) were obtained from a local pharmaceutical company Ronald Pharmaceutical Pvt. Ltd, Vadodara, Gujarat. Details of these compounds are given in Table 3.

Table 3: Details of pharmaceutical compounds

Diclofenac sodium Formula: $C_{14}H_{10}Cl_2NNaO_2$ Molar mass: 318.10 g/mol Melting Point: 284 °C CAS ID: 15307-79-6	
Ibuprofen Formula: $C_{13}H_{18}O_2$ Molar mass: 206.29 g/mol Boiling point: 157 °C CAS ID: 15687-27-1	

Nitrobenzene ($C_6H_5NO_2$), Tert butyl alcohol ($C_4H_{10}O$), Ethanol (C_2H_6O), 1, 10-Phenanthroline monohydrate ($C_{12}H_8N_2 \cdot H_2O$), Conc. Sulfuric acid (H_2SO_4), Conc. Hydrochloric acid (HCl), Potassium peroxymonosulfate (PMS) ($KHSO_5 \cdot 0.5KHSO_4 \cdot 0.5K_2SO_4$), Ammonium acetate ($C_2H_7NO_2$), Sodium sulfate ($Na_2SO_4$), Sodium chloride (NaCl), and Ferrous sulfate heptahydrate ($FeSO_4 \bullet 7H_2O$) were purchased from Loba Chemie Pvt. Ltd. Methanol (CH_3OH), Acetonitrile (C_2H_3N), Formic acid (CH_2O_2), and Phosphoric acid (H_3O_4P) was of HPLC grade and purchased from Loba Chemie Pvt. Ltd. Hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) and Titanium dioxide (TiO_2) were purchased from Suvidhinath Laboratories. Sodium sulfite (Na_2SO_3) and Sodium nitrite ($NaNO_2$) were of extra pure grade and purchased from Laboratory Rasayan and S D Finechem Ltd. respectively. The reverse osmosis concentrate (ROC) used in this study was collected from Bharucha Industry, located in Pandesara, Surat, Gujarat, whose influent was groundwater. Characteristics of ROC are given in Table 4. Distilled and deionized water was used throughout the study for dilutions and preparing aqueous solutions. All the chemicals and materials were used as received without any further purification unless stated otherwise.

Table 4: Characteristics of reverse osmosis concentrate

Parameters	***Average Values***
Total dissolved solids	3000 mg/L
Chloride	1600 mg/L
Sulfate	128 mg/L
Nitrate	4.5 mg/L
pH @ 27 ^{0}C	7.2 ± 0.3

3.2 Reactor configurations and experimental set-up

Outline of experimental work is given in Figure 5. All experiments were conducted in an undivided cell made up of acrylic and having dimensions: 200 mm x 120 mm x 70 mm. Experiments were performed at least twice to ensure the accuracy of data.

EO experiments: Electrodes were placed 40 mm apart on grooved Teflon spacers. The DSA (dimensionally stable anode) was prepared indigenously using the thermal decomposition method (Soni et al., 2020).Titanium plate (90% pure, Unisys Ortho., Ahmedabad) was base metal,

its surface was roughened with silicon carbide paper and plate was etched in 10% oxalic acid for 1 hour at 80^0 C. The precursor solution was prepared from $RuCl_3$, $SnCl_2$, and $SbCl_3$ salts, in IPA and 37% HCl solution. The freshly prepared precursor solution was applied on the titanium plate simply by brush. The solvent was allowed to dry for 5 minutes at room temperature and then it was oven-dried for 5 minutes at 80^0C to ensure solvent evaporation. It was then heated at 550^0C for 5 minutes to ensure the calcination of metallic salts. These steps 'applying precursor-drying-evaporating-calcination' were repeated after each coating for 15 to 16 times. After 15-16 coats of precursor solution, the plate achieved 1.5 mg/cm^2 weight gain, which ensured mixed metal coating. Then the plate was heated in a muffle furnace for 1 h to fix the film of metal oxides. The mol ratio of Ru:Sn:Sb in the coating was kept at 1:4:0.67. A steel plate was used as a cathode. The effective area (exposed to the solution) of electrodes was 100 mm x 93 mm. Electrochemical oxidation was performed in a batch mode. The current densities 5, 7.5, and 10 mA/cm^2; were applied and controlled galvano-statically by DC power supply (HTC instruments, Mumbai; range: 0-1 A and 0-30 V). All experiments were performed at neutral pH (7.5 to 7.8) and no further pH adjustment was done. The temperature was maintained at 27±2 ^{0}C throughout the experiments. Solution volume was 1 L, prepared by spiking diclofenac sodium (200 ppm in stock solution) in distilled water or RO concentrate to achieve 10 ppm DCF concentration. It was continuously stirred at 500 rpm. To check the effect of sulfate to chloride ratio, sodium sulfate and sodium chloride were added in distilled water so as to adjust sulfate to chloride mass ratios of S:C = 0.6:1, 0.85:1, 1.1:1, 1.35:1, and 1.6:1; keeping total dissolved solids 1000 mg/L. To avoid the effect of total salt concentration, these matrices were synthesized in such a way that total salt concentration remained 1000 mg/L. All the experiments were carried out at least twice.

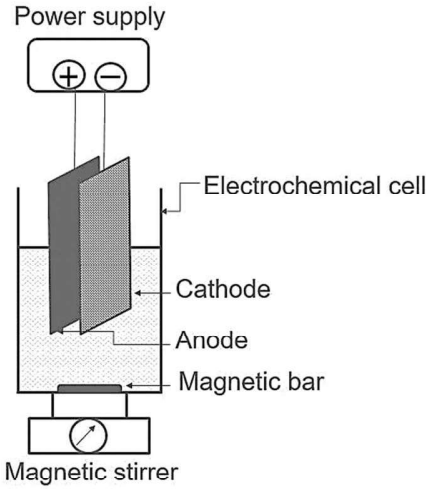


Figure 2 : Schematic view of the batch experimental setup for EO of DCF

EC/PMS experiments: The iron plate was used as a sacrificial anode and graphite plate was used as a cathode having dimensions 11 cm x 8 cm x 0.5 cm. Electrodes were placed 70 mm apart. The effective area of electrodes exposed to the solution was 9.5 cm x 8 cm. Experiments were carried out in batch mode as well as continuous mode. The temperature was maintained at 27±2 ^{0}C throughout the experiments. ***Batch experiments:*** At the start, 1 L of ROC spiked with 10 mg/L IBU was added in the acrylic cell. The cell was placed on the stirrer plate and the solution was stirred with a magnetic bar at 500 rpm. An initial sample was withdrawn and a predetermined amount of PMS was then added in the cell, at the same time current was supplied to start the reaction. DC power supply (HTC instruments, Mumbai; range: 0-1 A and 0-30 V) was used to apply and control current density. The reaction time for all batch experiments was 30 min. Figure 3 shows the batch experimental set up. ***Continuous flow experiments:*** Reactor was run in batch mode for half an hour and then feed was started at controlled flow. Initially, experiments were carried out at 2 L/h flow rate which gives 30 min retention time. Later for optimization purposes, the flow rate was increased to 2.5 L/h, 3 L/h, and 4 L/h which give 24 min, 20 min and 15 min RT respectively. Fifteen minutes buffer time was given to achieve steady-state condition and samples were started taken after this at every fifteen-minute for one hour and then the interval was increased to thirty minutes. Reactor was run in continuous mode for 3 h. Feed was stored in the reservoir which contained 10 mg/L IBU, 500 mg/L PMS dissolved in ROC. Feed was introduced at the bottom right corner as shown in Figure 4, and the outlet was on upper left side to ensure that there

is no short circuit of flow. The effluent was collected directly from an outlet for analysis.

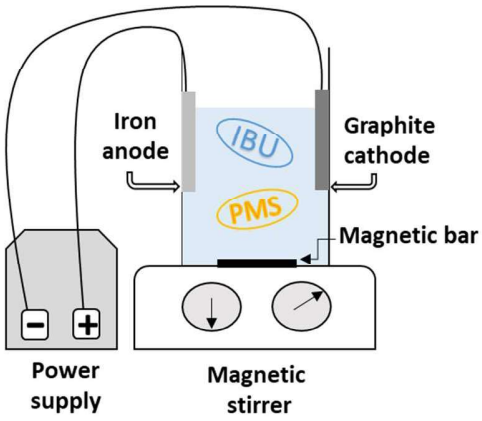


Figure 3: Schematic view of the batch experimental setup for EC/PMS of IBU

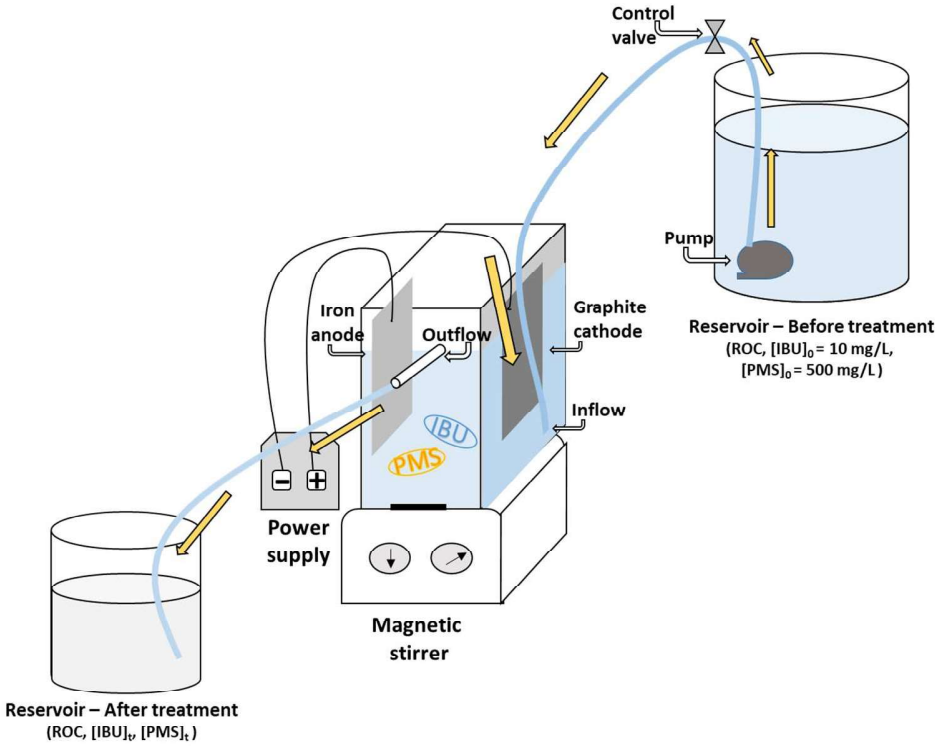


Figure 4: Schematic view of the continuous flow experimental setup for EC/PMS of IBU

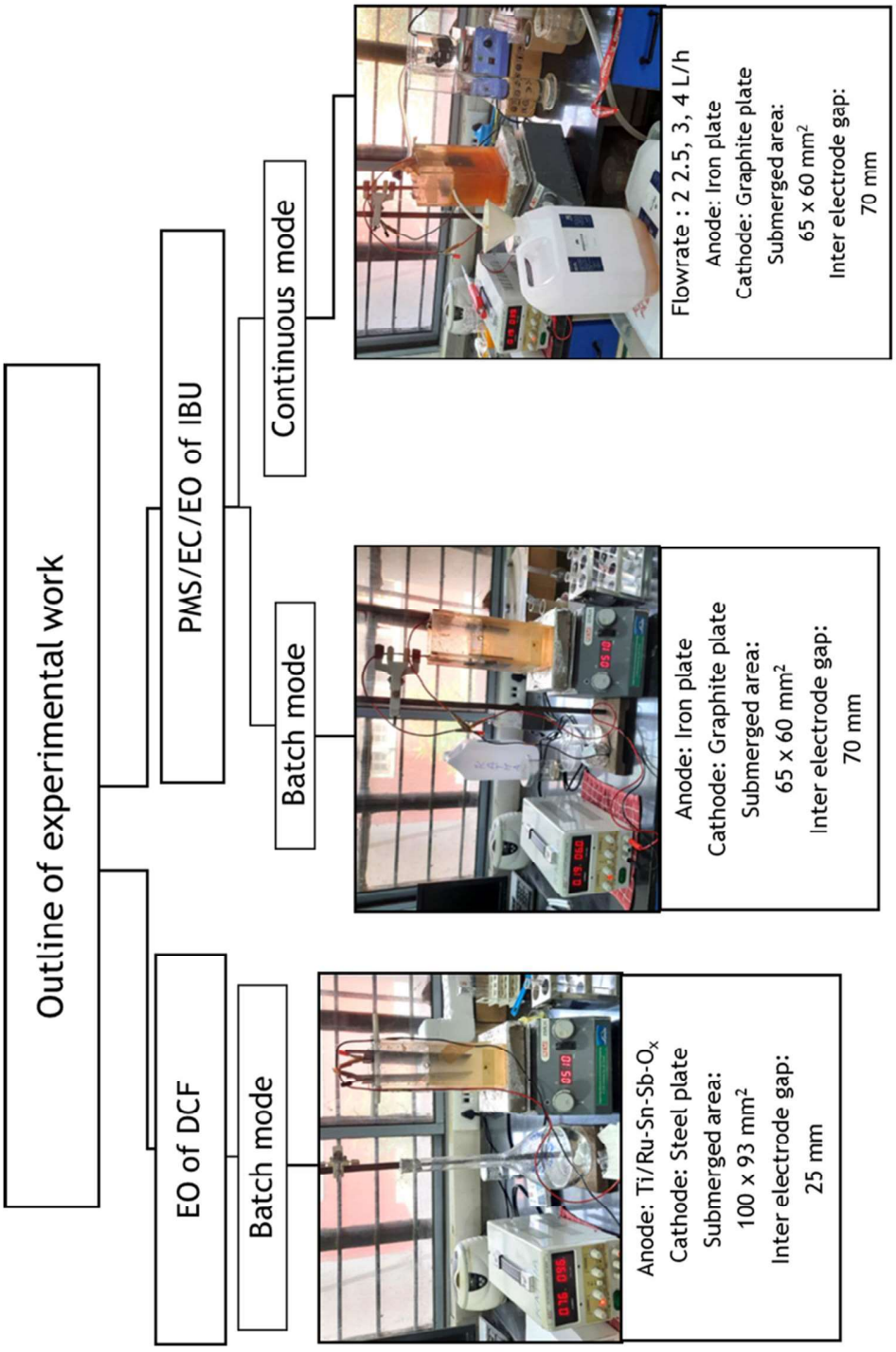


Figure 5: Outline of the experimental work carried out in the present study

3.3 Analytical procedure

For EO of DCF: To check the DCF concentration in EO treated samples, 2 mL sample was withdrawn at fixed time point and quenched with 2 mL methanol (HPLC grade). Sample was immediately mixed in vortex mixer for 30 seconds to ensure complete mixing. The concentration of diclofenac sodium and its intermediates during the electrochemical oxidation was analyzed by high-performance liquid chromatography (HPLC). The HPLC system consisted of liquid chromatography (Shimadzu, LC-2030 Plus) equipped with a C18 column (Shim pack GIST, 250 mm x 46 mm x 5 µm) and a UV-visible detector. The column temperature was kept at 25 ^{0}C. The mobile phase contained Acetonitrile (eluent B)/0.1% formic acid (eluent A) = 50/50 at a flow rate of 0.90 mL/min. All solvents were sonicated in an ultrasonic cleaner (make: Labman) for 10 minutes before analysis. The gradient program for this system was: 0.01 to 0.5 min from 50 to 0% Acetonitrile; 0.5 to 6 min from 0 to 100% Acetonitrile; 6 to 9 min from 100 to 0% Acetonitrile; 9 to 10 min from 0 to 50% Acetonitrile; finally returning to the initial conditions. The runtime was 15 min. The injection volume was 100 µL. UV-vis detector was used to scan absorption at 276 nm wavelength, and the data were analyzed with a LabSolutions software data acquisition system. The iodometric method was used to analyse reactive chlorine species (Palma-Goyes et al., 2016; H. Wang et al., 2019). Two drops of acetic acid were added in 5 mL volume of 50 times diluted aliquots to bring down pH 3 to 4. Two drops of KI solution (40000 ppm) were added to develop yellow color which was measured at 350 nm using UV-visible spectrophotometer (Brand: Shimadzu, Model: UV1800, Spectral Bandwidth: 1 nm).

For EC/PMS of IBU: To check the IBU concentration in EC/PMS treated samples, a 2 mL sample was withdrawn at the predefined time point. The sample was taken in a test tube containing 2 mL methanol (HPLC grade) to quench reactive species and to stop further IBU degradation. It was mixed instantly using a vortex mixer for 30 seconds to ensure rapid and complete mixing. To avoid iron flocs during IBU analysis in HPLC, samples were centrifuged for 15 minutes. The concentration of IBU during the reaction was analyzed by high-performance liquid chromatography (HPLC). The HPLC system consisted of liquid chromatography (Shimadzu, LC-2030 Plus) equipped with a C18 column (Shim pack GIST, 250 mm x 46 mm x 5 µm) and a UV-visible detector. The column temperature was kept at 25 ^{0}C. The mobile phase contained Acetonitrile (eluent B)/0.1% Phosphoric acid (eluent A) = 70/30 at a flow rate of 1.0

mL/min. All solvents were sonicated in an ultrasonic cleaner (make: Labman) for 10 minutes before analysis. UV-vis detector was used to scan absorption at 222 nm wavelength, and the data were analyzed with a LabSolutions software data acquisition system. The runtime was 12 min. The injection volume was 100 µL. Spectrophotometric methods were used to determine PMS, Fe^{2+}, and total Fe concentrations in the samples. For PMS determination in the sample, 1 mL of Titanium (+4) sulfate was added to 2.5 mL of centrifuged sample and mixed for 30 seconds in a vortex mixer. It was then allowed to cool for 10 minutes at room temperature, and solution absorption was measured at 410 nm using a UV-Visible spectrophotometer (Brand: Shimadzu, Model: UV1800, Spectral Bandwidth: 1 nm). Titanium (+4) sulfate was prepared by adding 2.5 g of Titanium dioxide in 250 mL of concentrated sulfuric acid, and this mixture was stirred at 150 rpm and heated at 100°C for 24 h (Rodriguez-Narvaez et al., 2020). For the determination of Fe^{2+} and total Fe, 1,10-phenanthroline method was used (Ling et al., 2017; Tamura et al., 1974). The concentration of Fe^{3+} was calculated by subtracting Fe^{2+} concentration from total Fe concentration.

For LC-MS analysis: The system consisted of a Water Acquity UPLC- H Class equipped with PDA and Acquity SQ detector. Chromatographic separation was performed on Waters X-bridge C18 Column (50*2.1 mm, 2.5 micron); Column temperature: 35 ^{0}C, Auto sampler temperature: 15 ^{0}C. Chromatographic analysis was carried out using isocratic elution and the mobile phase consisted of Acetonitrile (eluent B) and 0.1% formic acid (eluent A). Mobile phase gradient details are as follows : T = 0 min (97% A, 3% B) flow : 0.8 mL/min; T = 0.75 min (97% A, 3% B) flow : 0.8 mL/min; gradient to T = 2.7 min (2% A, 98% B) flow : 0.8 mL/min; gradient to T = 3 min (0% A, 100% B) flow : 1mL/min; T = 3.5 min (0% A, 100% B) flow : 1 mL/min; gradient to T= 3.51 min (97% A, 3% B) flow : 0.8 mL/min; end of run at T = 4 min (97% A, 3% B). The flow rate was kept 0.8 mL/min, run Time was 4 min, and UV Detection Method was PDA for wavelength range 200 to 500 nm. 10 µL of the samples were injected into the LC system. The analysis was conducted in both the electrospray (ESI) modes: positive and negative. The source working parameters were as follows: cone voltage: 30V and 10 V, capillary voltage: 3.0 KV, extractor voltage: 1 V, rf lens: 0.1 V, temperature of source: 120 °C, temperature of desolvation: 400 °C, cone gas flow: 100 L/hour, desolvation gas flow: 800 L/hour. The MS spectra were recorded in the range of 100 to 1000 m/z.

3.4 Quenching experiments for EO sample analysis

The initial concentration of diclofenac sodium in RO concentrate was 10 mg/L and applied current density was 7.5 mA/cm^2. During electrochemical oxidation, samples were withdrawn at fixed time intervals: Initial-0 min, 10 min, 25 min, 40 min, and 60 min; and quenched with different agents/techniques. Quenching agents were added more than the stoichiometric requirement to ensure the quenching of RCS produced at the given time point of sample withdrawal. Stock solutions of sodium sulfite and sodium nitrite were prepared of concentration 15 g/L and methanol (HPLC grade) was used as it is. In case of quenching with sodium sulfite or sodium nitrite, 0.25 mL of the quenching solution was added in 5 mL of sample. For methanol, 2 mL sample was collected in 2 mL methanol. Samples were vigorously mixed in vortex mixer to ensure complete quenching. For refrigeration, samples were preserved at 4 ^{0}C for 24 h. For alkaline conditions, sample pH was adjusted to pH>10 with drops of 1 N NaOH and supernatant was collected for analysis. Correlation test and Paired t-test were carried out for various data groups to support the results and conclusion of Quenching experiments.

3.5 Phytotoxicity testing

The Mung bean seeds (Vignaradiata L.) were used to carry out phytotoxicity test (Kumar et al., 2021; Stupar et al., 2020). The seeds were sterilized by 3% H_2O_2 for 1 min and seeds were washed thrice with distilled water. 100 seeds were placed in 100 mL glass beaker (sterilized) and soaked in 25 mL of treated solution. There were two controls: control 1 was distilled water and control 2 was RO concentrate. The seeds were soaked for 24 h and kept in dark and dry place. Then seeds were strained and incubated for 48 hours at ambient temperature (25 ^{0}C to 27 ^{0}C). The seeds were covered with sterilized glass petri-dish to ensure that there was sufficient moisture for germination. This test was carried out twice and average results are reported. The phytotoxicity was calculated using following equation 1 (Stupar et al., 2020):

$$Phytotoxicity(\%) = \frac{[(Radicle\ length\ of\ control) - (Radicle\ length\ of\ sample)]}{(Radicle\ length\ of\ control)} x\ 100 \quad (1)$$

3.6 Response surface methodology

For optimizing the factors affecting EC/PMS study, response surface methodology was used. A three-factor three-level Box─Behnken design (BBD) was applied to understand the effects of three independent variables on the EC/PMS process. The process variables were initial pH (A), initial PMS concentration (B), and current density (C); whereas the responses were % IBU removal and reaction rate constant for IBU (min^{-1}) degradation. The levels were coded as shown in Table 5. Total 17 experiments were designed as depicted in Figure 6. Experimental data were evaluated using software Design expert 11 (Version: 11.1.2.0).

Table 5: Coded independent variables and levels for BBD

Factor	Name	Units	Coded level		
			Low	Mean	High
A	pH	pH	-1 ↔ 5.5	0 ↔ 7.5	+1 ↔ 9.5
B	PMS	mg/L	-1 ↔ 100	0 ↔ 500	+1 ↔ 900
C	CD	mA/cm^2	-1 ↔ 0.525	0 ↔ 2.50	+1 ↔ 4.475

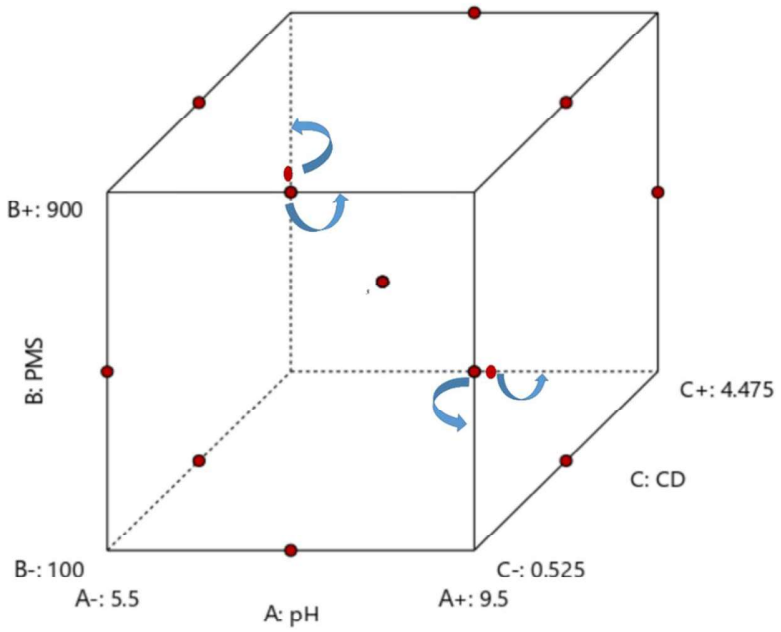


Figure 6: Design of experimental run for RSM